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Physiology News

Issue 118 / Spring 2020

Ecophysiology and climate change:

Shaping our understanding of animal form and function

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Welcome to the Spring 2020 edition of *Physiology News*

Introduction

News and Views

Events

Journal Insights

Features

Membership

Cover image: As part of a long-term project on the Svalbard reindeer, the research team equip a lucky female reindeer with a GPS and activity collar, as well as a subcutaneous heart rate monitor, to retrieve valuable data. They use a hot flask to keep the computers warm in the bitter Arctic cold. Learn more about their research on p.22. Photo credit: L Monica Trondrud.

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The Physiological Society

Physiology in the time of coronavirus

Keith Siew
Scientific Editor

Julia Turan
Managing Editor

Amidst the coronavirus crisis, physiology has been thrust to the fore with many awakening to the importance of physiology in understanding this disease, its treatment and prevention. Perhaps nothing exemplifies this more than the re-emergence of plasma therapy as a means to bestow passive immunity, a discovery awarded the first ever Nobel Prize in Physiology or Medicine in 1901 for its use as treatment of diphtheria.

In fact one of our authors, Michael Joyner, is leading such efforts with convalescent plasma clinical trials in the United States of America. His co-authored article with Paolo Dominelli (p. 41) about the oxygen dissociation curve in rare haemoglobin conditions is especially relevant, given the intense focus on COVID-19 patients' diminished blood oxygenation saturations, and the staggering demand for supplemental oxygen therapy and respirators. Here they argue that the study of such physiological curiosities could yield novel therapies for patients with reduced oxygen uptake in the lungs or storage capacity (i.e. anaemia).

Another topic normally bereft of attention is the importance of sex in physiological research. However, the need to understand sex differences has become all too clear with men much more likely to suffer more severe symptoms and higher mortality with COVID-19. As discussed by Natasha Karp *et al.* (p. 32), females (both human and animal) have historically been underrepresented in pre-clinical and clinical research, and while it is encouraging to see that two women were the first individuals to receive vaccines in US and UK phase I clinical trials, it remains imperative that all future studies properly incorporate sex to ensure the efficacy and safety of treatments for both men and women.

In the eyes of many, COVID-19 has strengthened the case for open access (p. 12), and there has been a flurry of pre-prints hoping to expedite dissemination of the latest breakthroughs. Indeed, The Society journals have made all research related to COVID-19 freely accessible in the furtherance of the public good, in line with many other scientific publications. Although the sense of urgency is warranted, the breakneck pace of research and immense pressure to produce positive results is not without concern. We must temper our desire to cut corners and avoid the trappings of poorly designed experiments, particularly in the pre-clinical setting. A welcome refresher from Michael Festing (p.14), reminds us that designing rigorous statistically valid experiments is paramount, if we are to avoid producing irreproducible studies that give false hope and could ultimately cost lives. Sadly this is not the only threat, as tragedy often begets opportunity, and those less honest driven by non-altruistic tendencies may seize upon this moment to make a name for themselves. Elisabeth Bik, who we interviewed in this issue, will be on their case (p. 10). She keeps an eye out for fraudulent data, and seeks to expose authors and publications, to encourage ethical publishing. Philip Lewis further explores "science as a human endeavour" expanding on the discussion about sources of bias and flaws in scientific publishing in a review of a book by Member Gareth Leng and his son Rhodri Leng, *The Matter of Facts* (p. 48).

This pandemic has left little of our lives unchanged. We are in a marathon. The constant mental battles and worry are tiresome (see "Mental health in academia", p. 22) and the struggle to maintain routine presents a real challenge to the health of our biological rhythms (see "The bustling world of hormone dynamics", p. 44). Those particularly in our thoughts are clinical colleagues who are having to endure grueling hours and ever-changing shift patterns, all while being expected to maintain peak performance (see "Time to win gold" p.36). Also we, like I'm sure many of you reading this, have had a steady stream of family and friends ask for our takes on the scientific and medical advice. But for those without such ties, continuous education of the public about the latest science has never been more important and this issue showcases two excellent examples that will hopefully inspire you to involve yourself in public engagement: Simon Watt writes about his Society-funded podcast Level Up Human (p.47), and Lizzie Burns about her "Amazing Body" drawings that aim to help hospital patients relax and also educate them about the beauty of their microscopic inner workings (p. 46).

Perhaps one silver lining of this situation is the positive environmental impact. Liv Monica Trondrud and Alina Evans' article (p. 26) about ecophysiology, the study of animal form and function, reminds us of the importance of this field in understanding how human actions impact animals. Additionally, our exciting upcoming virtual early career conference Future Physiology will focus on climate change, consisting of a week of oral communications, e-poster sessions, and professional development workshops. Please visit **physoc.org/futurephysiology2020** to register.

Finally, in non-coronavirus related news, we are incredibly excited to announce that from this issue onwards Physiology News will be published under a Creative Common licence, specifically CC BY-SA 4. For more information, visit **physoc.org/magazine**. Under this licence, anyone can use the text and images in the magazine provided they give appropriate credit, provide a link to the licence, indicate any changes they have made, and distribute it under this same licence. We thought this change to be important on principle - the more Members who reuse our materials, in the classroom or otherwise, the better.

Looking back to previous issues, Society staff member Mary Arbuthnot has worked tirelessly for months converting the PDF version into an online archive with articles on individual web pages, that is indexed and searchable. Each of these articles now has a DOI (digital object identifier), making it citable.

This project has been completed all the way back until issue 43 and will be picked up and finished off later this year. The print edition will also include DOIs beginning with this issue. The magazine's permanent DOI prefix is: 10.36866 and we are using the following format for generating DOI suffixes: **https://doi.org/10.36866/pn.issue#.page#** (with a,b,c) being used as necessary after page number for instances of multiple articles on the same page.

We welcome your submissions and suggestions for our upcoming Education Special Issue. Our audience is primarily undergrad and postgrad educators. We aim to cover pedagogical research, resources, and personal stories. We are particularly interested in how COVID-19 has impacted educators.

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The Society's response to the COVID-19 pandemic

Bridget Lumb

President, The Physiological Society

As I write this, we are living in an unprecedented period with the COVID-19 global pandemic having a profound effect on every aspect of how we live and work. Scientists across the world are having to adapt quickly and I have heard many inspiring stories about the way people are dealing with this crisis.

It is more important than ever to come together as a scientific community as we work to tackle this pandemic. The Society quickly formed a COVID-19 advisory panel and launched our "Questions from the Frontline" initiative, which seeks to provide physiological insight to clinicians dealing with patients. We have made research articles in our scientific journals that are relevant to COVID-19 freely accessible and we will continue to do this over the coming weeks and months.

The Society is also adapting our activities and resources so that we can continue to support our Members during this challenging time. We have launched our online COVID-19 hub on our website which contains a growing range of resources to assist Members, including

- Teaching materials to assist with transitioning online
- Professional development webinars to enhance your career
- Mental health training
- Grants to support science communication
- Advice to help you submit papers to journals
- Advice on returning to lab work while maintaining social distancing

This list continues to be added to, so please do visit the COVID-19 hub and let us know if there are resources you would find useful.

I am pleased that by quickly adapting to working from home during the lockdown, The Society's staff have been able to continue to provide day-to-day Member services, with phone calls and emails answered as normal.

The pandemic has unfortunately necessitated the cancellation of our face to face 2020 events, including Europhysiology in Berlin, which was going to feature our annual conference. The Europhysiology Organising Committee unanimously agreed that, although the situation is currently improving, holding a large international conference in early September is unrealistic.

Our Europhysiology series of conferences was established to bring physiologists from across Europe together to celebrate science and we still plan to hold our next conference in Copenhagen, Denmark in September 2022 and we look forward to seeing you there.

Despite the current restrictions, we are determined to continue providing physiologists with opportunities to hear the latest science and for professional development. Therefore, we are seeking to move as many of our events as possible online, as well as rescheduling others.

Our conference Future Physiology in July will be run as an entirely virtual event between 6-10 July comprising talks, workshops and ePoster sessions for delegates to engage with remotely. This innovative way of connecting physiologists from around the globe will provide an opportunity for early career researchers (ECRs) to share new research, gain practical skills, and network with peers. This year's conference is called *Physiology in a Changing Climate: The Interdependence between Physiology, Behaviour and the Environment*. The conference will be tailor-made to give ECRs the experience and enthusiasm to take the next steps in their career.

As the pandemic progresses we will continue to monitor and review our programme of activities to ensure we are doing our very best to support the physiology community at this time and I would encourage you to regularly check our website for updates on events planned for later in the year. I looking forward, more than ever, to next year's annual conference in Birmingham!

As I approach the last few months of my Presidency, it seems a good time to reflect on the last couple of years. During this time we have held a fantastic main meeting in Aberdeen, hosted our first public event at the Royal Institution with NASA astronaut Jim Pawelczyk, and launched our first two parliamentary reports in our history.

It's been a busy and rewarding time for The Society and at the heart of everything we do is our membership. I am proud that we represent the largest network of physiologists in Europe: from early career through to Nobel Prize winners, we embody the full spectrum of our discipline. I am delighted that our membership numbers continue to grow but we must never stand still. We are currently undertaking a membership review to listen to current and prospective Members about how we can make The Society – *your* society – the best it can be. We want to explore how we can support physiologists in their careers, enhance their networks and continue to be a hub for world-changing science.

One of the real strengths of physiology is the breadth of the discipline, and we are actively working to engage every community. For example, our recent Sport and Exercise Science (SES) report has been warmly received by SES departments across the country. We have recently held policy events in Cardiff and London and we have strengthened our Society Representative network, with each institution's representative now searchable on our website.

The next few weeks and months will be challenging for everyone as we face this crisis. The Physiological Society will continue to support you as we move through this period.

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A recipe for confusion and error: Misleading terminology for reporting of oxygen concentration in cell culture media

Michael T Kane
NUI Galway, Ireland

Blood equilibrated with air under normal laboratory conditions contains about 20% O_2 (v/v). As every first-year student in physiology learns, given the very low solubility of oxygen in aqueous solutions, this concentration of oxygen in normal arterial blood is only possible due to the oxygen-carrying power of haemoglobin.

Thus reports in some publications (see below) of levels of up to 20% oxygen in haemoglobin-free biological fluids such as cell culture media are both meaningless, in the absence of units, and grossly misleading. The true figure is of the order of 0.4% O_2 (v/v) based on the fact that solubility of oxygen in plasma is given as 2.09 ml per 100 ml plasma at 38°C and 1 atm (760 mmHg) pressure of oxygen in the gas phase.[1] Oxygen is both essential to cells for their viability and energy production but is at the same time dangerous to those same cells because high concentrations can lead to the production of reactive oxygen species and free radicals, which damage cells. This is referred to as the Oxygen Paradox of Davies.[2] Therefore, it is of vital importance that the terminology used to report the oxygen concentration in cell culture be always unambiguous and correct. This letter provides examples of errors in this context and explains how such egregious errors may have arisen.

In a 2012 paper on chondrogenic cell culture in *In Vitro Cellular & Developmental Biology–Animal*, one oxygen concentration in the culture medium is given as 18.99%. In another paper on human trophoblast culture in *Placenta* in 2013, it is stated that the concentration of oxygen resembled that in the ambient atmosphere. Another paper on HeLa cell culture in *Cell Biology International* in 2016 reports dissolved oxygen concentrations up to 18%. This kind of error is not confined to culture studies. A paper on the oxygen concentration of human ovarian follicular fluid in *Human Reproduction* in 1997 reports "average percentage dissolved oxygen" figures of 1.7 to 4.2. A paper on cattle follicular fluids in *Theriogenology* in 2008 reports follicular fluid oxygen concentrations of 6.9 % and 7.3%.

Even in an otherwise excellent and very useful paper entitled "Dissolved oxygen concentration in culture medium: assumptions and pitfalls", there is the comment "in this study, dissolved oxygen levels in culture medium maintained in standard culture conditions (18% O_2) measured 18%".[3]

How has this erroneous terminology crept into current mainstream literature?

A minor reason is probably the general custom of referring to gas phase concentrations in gas mixtures without units e.g. 5% CO_2 and not 5% CO_2 (v/v). A major reason is the failure to realise that oxygen is very poorly soluble in aqueous media. However, another major reason is that much of the instrumentation generally used for measurement of dissolved oxygen in culture media or biological fluids was not originally designed for this purpose. One such device is the Jenway DO_2 oxygen meter.[3] Email correspondence with the suppliers of these meters informed me that "the Jenway DO_2 meters were originally developed for determining the quality of environmental water systems... The meters are able to display either % oxygen saturation or % air saturation and are calibrated according to the parameter required."

It is clear from information on the websites of other manufacturers and some email correspondence with them that most, if not all, the oxygen sensor probes available are based on a polarographic electrode or alternatively a fibre-optic device that measures PO_2 which can then be translated into other units including % oxygen saturation or % air saturation but NOT usually % oxygen v/v. Thus, when papers like those cited above say that the medium has an oxygen concentration of 5%, this does not mean that the medium contains 5% v/v dissolved oxygen. Rather, it means that the medium contains the same concentration of oxygen as it would if it were fully equilibrated with a gas phase of 5% v/v oxygen, so that the PO_2 of the gas phase and the fluid are exactly the same i.e. the % oxygen saturation is 5%. Based on the oxygen solubility in plasma[1] the true value of the % oxygen concentration (v/v) for 5% oxygen saturation is about 0.10% i.e. about 1/50 of the oxygen saturation value. Note that in saying v/v or vol % it is obviously understood that one is using the same volume unit for both numerator and denominator.

A crucial point or much ado about nothing?

It may seem that this question of terminology is like Shakespeare's play *Much Ado About Nothing*. However, this is not correct. Firstly, in specifying culture medium levels for oxygen, which is essential for cell function but potentially toxic, the terminology used should be, as stated above, unambiguous and correct. At a time when the most recent Nobel Prize in Physiology or Medicine has been awarded to Kaelin, Ratcliffe and Semenza for their discovery of how cells sense and adapt to oxygen availability, it would seem more essential than ever that authors and journal editors report oxygen concentrations in biological fluids appropriately.

Secondly, the misuse of the terminology is not producing minor errors, it is suggesting values that are out by a factor of about 50 and clearly impossible with normal incubator gas pressures and gas phase oxygen concentrations.

Thirdly, reporting values as % oxygen saturation as used in reference 4 would be correct.[4] However, this is a clumsy terminology for cell culture and since gas diffusion always takes place along a partial pressure or tension basis, it is preferable that measurements should be made and reported in partial pressure units such as mm Hg. Some of the oxygen sensors, but not all, can be set up to report readings in partial pressure units (mm Hg, kPa).

"Reports in some publications ... are both meaningless, in the absence of units, and grossly misleading"

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Reports of The Society's recent committee meetings

The purpose of these short updates is to keep you informed about the work of our committees. The following summaries detail the meetings of the past few months.

Council

December 2019

The President, Bridget Lumb, thanked Trustees for their attendance at the General Meeting, which took place on 10 December 2019 at Hodgkin Huxley House and confirmed that, with 100% vote in favour, the new Articles of Association had been passed as a special resolution. The document would be formally filed with Companies House and the Charity Commission by BDB Pitmans. She noted some issues had arisen through discussion that would be taken forward with the Chair of the History and Archives Task Force. However, the completion of such a thorough review and implementation of the new Articles and Regulations would, going forward, allow Trustees to be confident in the robust governance structures and concentrate more on outward-focusing strategic priorities.

The Head of Policy and Communications reported on the new website data following 6 months in operation. Overall, the platform had been positively received and, following feedback from a focus group of Members and a subsequent meeting with the design agency, certain areas would be reviewed and reformatted to increase engagement. He noted that clear Key Performance Indicators had been set for each section and Council requested quarterly comparison data against the old website's performance.

The Chair of the Grants Review Task and Finish Group, Prem Kumar, provided a paper and gave a presentation that set out the recommendations of the group. Council received the work of the group positively and noted the thorough consideration that had been given to the overall offering of grant provisions in alignment with the strategy. Overall, it was considered a refreshing and forward-looking roadmap. Council agreed that a Grants Implementation Task and Finish Group chaired by Prem, which would include two Trustees, should be established to determine the finer details of the scheme. This implementation group would report to a future meeting for final approval of Council.

The Deputy Chair of the Membership Categories and Journeys Review Task and Finish Group, Guy Bewick, reported the findings of the group to Council. He noted the overarching aim of the review had been to develop a more inclusive Society, attracting Members from both inside and outside of the academic career path, and ensuring greater retention by providing appropriate career development opportunities. Council supported the principles of the recommendations but agreed that further research and input from the membership was required before approval could be granted. Council agreed to launch a series of roadshows throughout the UK to engage with the membership at their institutions over the next 12 months. The goal would be to work towards joining the revised membership categories and benefits, and grant schemes, to launch together.

"The overarching aim of the [Membership Categories and Journeys Review Task and Finish Group] had been to develop a more inclusive Society, attracting Members from both inside and outside of the academic career path, and ensuring greater retention by providing appropriate career development opportunities"

The Honorary Treasurer (HT), Frank Sengpiel, presented the 2020 budget, which had been approved by Finance Committee for recommendation to Council. Trustees considered the summary budget overview, which showed the 2019 budget, final forecast and the proposed 2020 budget along with a financial framework for the 2020 – 2022 period. The budgets had been constructed on a baseline/business-as-usual basis before any strategic project costings. Strategic investment projects would be funded from the Reserves, as detailed in the new Reserves Policy adopted by Council at CL19.01. However, the HT highlighted the potential risks when newly invested strategic programmes transition into ongoing services as this was where unsustainability could occur. He noted the importance of a timely review of ongoing projects to ensure they were still delivering the desired cost/benefit and to assess whether they should be replaced by any new strategic initiatives that had the potential to transition "business as usual." Council approved the 2020 budget.

To note: The Coronavirus pandemic will affect some of the activities The Society had planned to deliver in 2020. However, the Trustees are regularly reviewing the situation and working with the Senior Management Team to assess the impact of this ongoing crisis and will keep members updated as decisions are made.

Education, Public Engagement and Policy Committee

March 2020

The Education, Public Engagement and Policy Committee (EPEP) met via video conference on Monday, 30 March and was chaired by Sarah Hall, Cardiff University. The Committee's first item was to discuss the outcomes of the first phase of the project into career paths for those studying physiology and physiology-related courses at university. It was noted that recruitment of students will be a particular priority beyond the coronavirus because of the financial risks

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associated with low student numbers; thus the second phase of the project will focus on how to most effectively communicate the report's findings around employability to students, Society Representatives, careers advisers and those involved with university open days.

The Committee also discussed projects related to the teaching of physiology, with a particular focus on the impact that COVID-19 would have on the significance of lecture capture, live-streamed lectures, virtual practical demonstrations and recognition for those educators driving innovation in this space. It noted the importance of sharing resources during this time and recommended a role for The Society in facilitating this. The Committee also received an update on proposed education symposia at both upcoming conferences, Europhysiology 2020 and Physiology 2021.

The Committee noted the success of the recent webinar programme hosted by The Society in collaboration with the British Society for Immunology. It agreed that this was an excellent starting point from which The Society could learn about how to engage with Members remotely during coronavirus lockdown and was positive about proposed plans for this period.

The Committee also heard about plans to coordinate with *Advances in Physiology Education* and about how The Physiological Society is supporting projects financially that aim to reach the general public with information about the importance of physiology. The committee received a report from the Scientific Editor and the Managing Editor of *Physiology News* to update on recent activity and plans for the upcoming Education Special Issue. The Committee also fed back on the work of the Policy team regarding recent events coordinated with devolved parliaments of the UK and future projects looking at building a base of evidence in support for funding of physiological research in the run-up to the UK Government's Spending Review later in the year.

Finance Committee

November 2019
In its final meeting of 2019 the Finance Committee (FC) reviewed the Q3 Management Accounts and noted the year-end forecasts against actuals. They discussed areas of activity that had impacted budgeted income variances and acknowledged The Society's position on changes to the Travel Grant policy.

FC received the 2020 – 2022 budget proposals for recommendation to the Board. The 3-year financial plan highlighted The Society's financial sustainability on a business-as-usual basis and did not include any additional strategic projects that would be funded by the Strategic Investment Fund.

FC received an update on 2019 audit planning that was due to commence in March 2020 and noted progress on a tender for a proposed change of audit firms from the 2020 audit.

January 2020
The Finance Committee received a presentation from its investment management firm, Cazenove Capital Management, on the 2019 portfolio performance and 2020 outlook. The portfolio had grown by 14.4% in the 12 months to Q419 and had returned 1.9% in the last quarter while the long-term inflation target (CPI+4.0%) equated to a return of 1.1%.

FC received the 2019 Q4 Management Accounts and noted the projected figures from The Society's publishing partner Wiley. They acknowledged that it was a requirement under charity law to consider the valuation of the investment property elements of HHH each year, thus The Society had sought an informal assessment of the market from Mann Smith, Chartered Surveyors.

Consequently, the investment property value was reduced from £3,600k to £3,414k.

FC received the Audit Planning Report, which set out the audit process for Trustees' consideration. Based on their knowledge of The Society, the audit firm haysmacintyre, had concluded that there were no additional significant risk areas.

Following a tender process and interviews, FC recommended the appointment of Buzzacott LLP as the Society's auditors from 2020.

Finally, FC received and reviewed the Key Risks Report and Risk Register.

The Society has a wealth of experts working in areas related to coronavirus (COVID-19). Our communications team is working on delivering reliable, evidenced scientific insight on this disease through social media, video and our media work. To watch the blogs and watch the animations visit **physoc.org/coronavirus**

Pregnancy and coronavirus

In pregnancy, women's immune systems are naturally weakened so that their bodies don't reject the developing baby. Does this mean they aren't able to fight off coronavirus as well as someone who is not pregnant? We interviewed Physiological Society Member Erica Watson, Lecturer in Reproductive Biology, at University of Cambridge, about the possible increased susceptibility of pregnant women to severe symptoms such as pneumonia, and what this might mean for the developing baby.

bit.ly/2T04Ob6

How does COVID-19 affect our lungs?

When we become infected with COVID-19, this may damage our respiratory tree and specifically the tiny air sacs at the tips where gas exchange occurs. Debby Bogaert, Scottish Senior Clinical Fellow and Honorary Consultant in Paediatric Infectious Diseases at the University of Edinburgh, explains how inhibiting gas exchange affects our physiology.

bit.ly/2y38zFu

Can stem cells help fight coronavirus?

Physiological Society Member Georgina Ellison-Hughes, Professor of Regenerative Muscle Physiology at King's College London discusses her work on a new technique to potentially improve the outcome of patients with COVID-19 that transplants mesenchymal stem cells. The idea behind her technique is the ability of mesenchymal stem cells to prevent the dangerous cytokine storm, a dangerous overreaction of our immune system.

bit.ly/2zx77eL

Physiology Feed continues on page 13

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The science and art of detecting data manipulation and fraud: An interview with Elisabeth Bik

Julia Turan
Managing Editor, *Physiology News*

How big of a problem is data manipulation and fraud in biomedical science?

It is hard to make a good estimate about the percentage of papers with manipulated data. In my search of 20,000 biomedical papers that contained western blots (photos of protein gels stained with an antibody to analyse that protein's expression) I detected image duplication in about 4% of the papers.[1] Some of those duplications could be simple errors, but about half of those papers contained shifted, rotated, mirrored, or manipulated duplicates, which are more suggestive of an intention to mislead. So based on that study, we might conclude that 2% of those papers might contain intentionally duplicated photos. But the percentage of manipulated data, so not just looking at photos but also considering tables and line graphs, might be much higher. It is very hard to detect falsified or fabricated data in a table unless you compare the original lab book notes to the published data. Therefore the true percentage of data manipulation is probably much higher than 2%.

Which data are prone to the most manipulation/fraud?

All data. For me, it is easiest to detect duplications in photos, but I sometimes find unrealistic data in tables as well. For example, I found tables in which the standard deviation of dozens of values was always around 10% of the mean value they represent. That is not realistic for biological data, which is usually much more variable.

How, if at all, are journals/institutes/governments dealing with it?

Most journals that are part of the large scientific publishing houses scan for plagiarism, which is a form of research misconduct. Several journals, such as *Nature*, *PLOS One*, and *Journal of Cell Biology*, have recently implemented more strict guidelines for photographic figures, such as specifically prohibiting cloning, stamping, splicing, etc. And some journals are starting to better scrutinise images in manuscripts sent to them for peer review, as well as asking authors to provide raw data.

Institutes have not been very responsive to allegations of misconduct. Most institutes in the US will provide some classes on misconduct, but when it comes to actually responding and acting upon whistleblowers' reports, they tend to underperform. Most of the misconduct cases are swept under the rug and, very often, the whistleblower is the one who is fired, not the person accused of the misconduct. Kansas State University ecologist, Joseph Craine, and Johns Hopkins University statistician, Daniel Yuan, were both fired for being whistleblowers,[2,3] while Eleni Liapi of Maastricht University lost access to her lab and servers before being asked to quit,[4] and Karl-Henrik Grinnemo's career was severely damaged.[5]

What is an image duplication detective and how did you get into it?

I started this work around 2014, when I investigated a PhD thesis with plagiarised text in which, coincidentally, I spotted a duplicated western blot. I realised that this might also happen in published science papers, so I started scanning papers that very evening. Immediately, I found some other cases, and I was fascinated and shocked at the same time. Since then, I have scanned 20,000 papers in a structured way, so from different journals, different publishers, and different years. After the publication of our 2016 *mBio* paper together with Ferric Fang and Arturo Casadevall,[1] I kept on doing this work. About a year ago, I left my paid job to do this work full time.

There are several ways I scan papers, but I mostly follow up on leads that other people send me ("Can you please check the papers by Prof. X because we all suspect misconduct?") or on groups of papers from the same authors that I found earlier. Image misconduct appears to cluster around certain persons or even institutes.

What has been the reception to these activities in terms of resistance or support from the community and powers that be?

The reception has not been very warm, as you might imagine. Journal editors were possibly embarrassed and perhaps even overwhelmed when I started to send them dozens of cases of papers with duplicated images. Over half of the cases I sent to them in 2014 and 2015 have not been addressed at all, which has been frustrating. Some editors refused to respond to me and others have told me that they did not see any problems with those papers.

Institutes to which I reported sets of papers by the same author(s) have mostly been silent as well. But in the last couple of years, the tide has been changing, and I am starting to see more and more journal editors who are supportive and are actively trying to reject manuscripts with image duplications, before they are published.

How do you detect image manipulation, and are there resources to help automate detection or learn how to do it?

I scan purely by eye. Having scanned probably over 50,000 papers by now, I have some experience on which types of duplications or manipulations to look out for. For complicated figures with lots of microscopy panels I use Forensically[6] but that can only detect direct copies, not anything that has been rotated or zoomed in/out.

"There will always be dishonest people, and photoshopping techniques are getting better and better, so it is unrealistic to think we can catch all of these cases [of image manipulation] during peer review, even with detection software"

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There is no good software on the market yet to screen for these duplications, but there are several groups working on automated approaches, with promising results.[7,8,9]

What are some of the most common types of manipulations? How are these done?

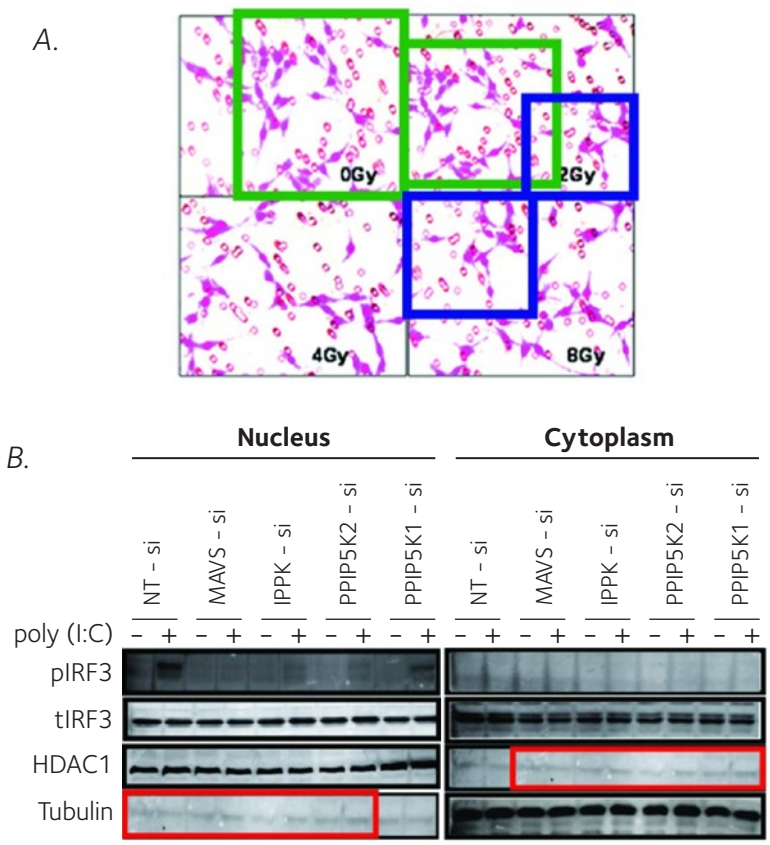
The most common ones are overlapping microscopy images. These are two photos that represent two different experiments, but that actually show an area of overlap, suggesting they are the same tissue. Another very common type is western blots that are shifted or rotated to represent two different experiments. These two examples are not photoshopped, but manipulated in the sense that the photos are somewhat changed (shifted, mirrored, rotated) to mislead the reader. True photoshopped images, where parts of photos are cloned or copy/pasted into other photos are quite common among flow cytometry images.

How have social media and crowdsourcing helped in this endeavour (i.e. Twitter, or your blogs Microbiome digest and Science Integrity Digest, etc.)?

They are helping in making people better peer reviewers, and more critical readers of scientific papers. I use Twitter to show examples of duplications, so people are more aware of them, and can find these cases in the future. PubPeer.com is a website where individual papers can be discussed and flagged for all kinds of concerns. ScienceIntegrityDigest.com is meant for more reflective blog posts, or to describe patterns among scientific papers that cannot be spotted by looking at individual papers, such as the paper mill of over 400 papers that we recently discovered.[10]

What are your hopes for the future of image duplication, manipulation and fraud detection and handling as a community?

There will always be dishonest people, and photoshopping techniques are getting better and better, so it is unrealistic to think we can catch all of these cases during peer review, even with detection software. But I hope we can take some of the pressure off scientists that feel driven to publish at any cost. Scientific papers are the foundation of science, but it is unrealistic to ask graduate students, postdocs and assistant professors to publish X number of papers with a combined impact factor of Y before they can graduate or get tenure. Good science takes time, often fails, and never keeps to imposed deadlines. If we measure good science by the wrong output parameters, we put too much temptation onto people to cheat.



Examples of duplication with repositioning *A*. Although the panels represent four different experimental conditions, three of the four panels appear to show a region of overlap (green and blue boxes), suggesting that these photographs were actually obtained from the same specimen. *B*. Western blot panels that purportedly depict different proteins and cellular fractions, but the blots appear very similar, albeit shifted by two lanes (red boxes).

What is next for you and how do people keep track of your fascinating activities?

I am not sure yet! I have so many interesting leads to follow that I will probably be busy uncovering "clusters" of misconduct for the next couple of years. But I also hope I will be less regarded as a pair of extraordinary eyes with a Twitter account, and more as a real scientist who wants to improve science. I hope there will be a place at the table for me with institutes and publishers to talk about better ways to detect and decrease science misconduct. You can always follow me on Twitter at **@MicrobiomDigest**.

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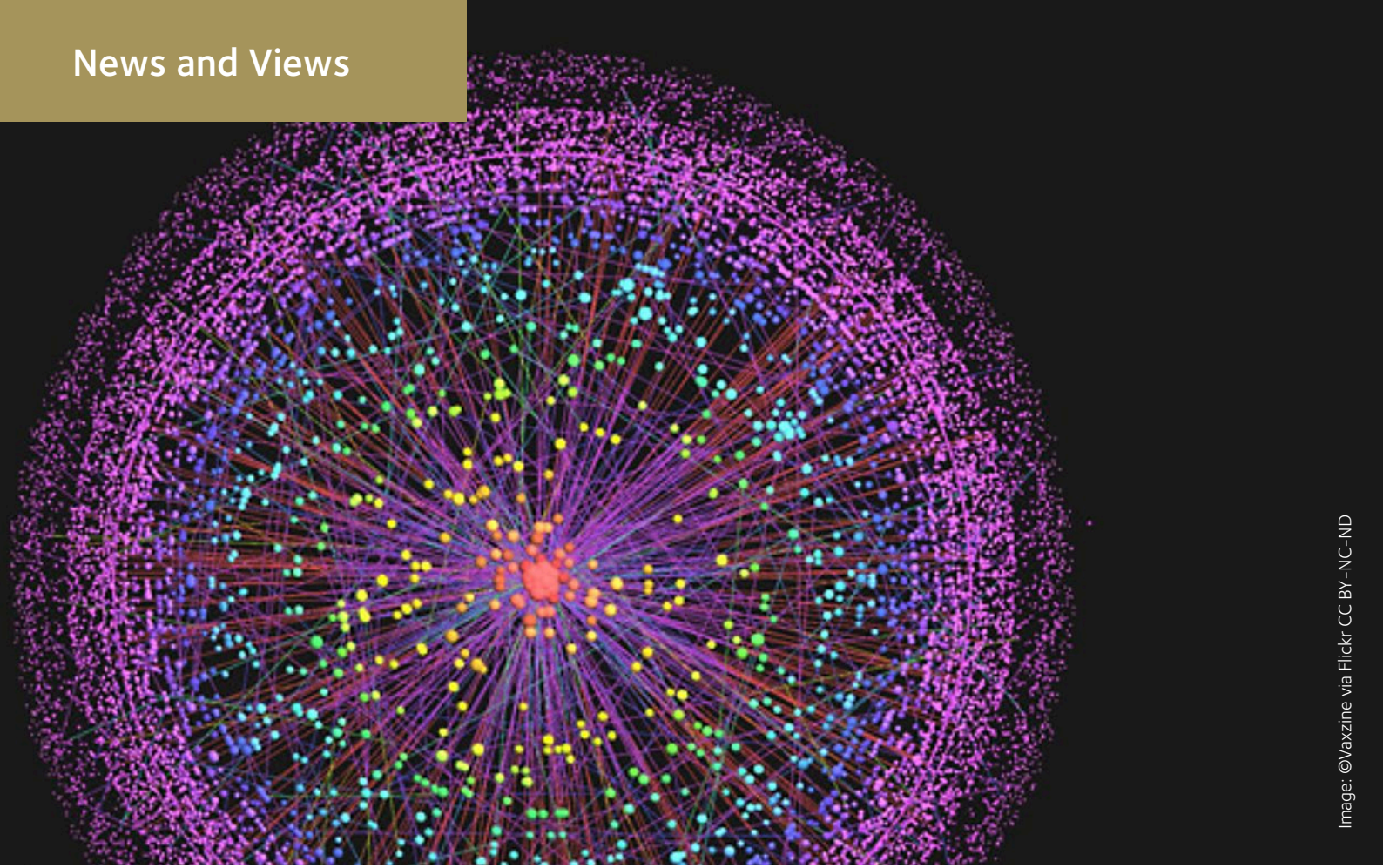

Transformative Agreements and the immediate future of Open Access

Alex Stewart

Deputy Managing Editor,
The Physiological Society

Plan S, the open access movement driven by some of Europe's major research funders, has recently encountered some notable hurdles in its path. In March, its figurehead, Robert-Jan Smits, left his role as the European Commission's Special Envoy on Open Access. Shortly thereafter, the deadline for publishers' total compliance to the Plan was delayed by a year to January 2024. This delay was caused by considerable consternation at some of the initiative's more controversial requirements and was announced alongside certain concessions.[1] Compromises included the acceptance of hybrid journals (subscription journals that offer immediate open access of individual articles at a cost to the authors) as compliant until 2024, provided such journals can demonstrate their commitment to becoming fully open access by this deadline through the means of so-called transformative agreements.

Transformative agreements are contracts between institutional library consortia and publishers that facilitate the transition of scholarly journals' business models to open access. These agreements work by converting current institutional subscription spend into open access publishing spend. Estimates suggest that, annually, €7.6 billion is spent globally on journal subscriptions and 2 million articles are published.[2] Libraries foot the majority of this bill, contributing significantly to the generous profit margins enjoyed by many publishers.[3] Proponents of Plan S, and open access in general, suggest that this total spend could be cut to €4 billion in an entirely open access world, assuming article processing charges (APCs) are capped at €2,000. This cap is somewhat lower than current APCs imposed by many of science's most highly regarded journals. The economics of open access publishing depend heavily on the rejection rate of the individual journal.

While there currently exists no fixed model of transformative agreement, many are being experimented with. The key breakthrough was the agreement struck between Wiley and Projekt DEAL (a consortium of German research institutions and funding agencies). The discussions around this 3-year contract centred on a Publish-and-Read model. The "publish" part of this deal grants immediate, free open access to all publications authored at institutions belonging to the consortium. Instead of costs for this deal being calculated on a subscription fee for reading, the consortium pressed for a "fair costing" based on the volume of papers published in Wiley journals by its researchers. The two parties agreed on a fee of €2,750 per article for up to 10,000 articles yearly – a total derived from the historical spend by the participating libraries on access to Wiley journals. The "read" part of the agreement provides the consortium's libraries with free access to the whole of Wiley's subscription research collection. In summary, DEAL institutions pay the APCs for up to 10,000 articles per year from their affiliated researchers to be published as open access in Wiley journals and get subscription to Wiley journals for their institutions included.

Under the terms of this agreement, individual authors no longer need to worry about finding funds to pay APCs for immediate open access publishing. They also retain copyright to their work. The transformation is essentially cost neutral both for the consortium members and for Wiley. The agreement is also considered compliant with Plan S, with the hybrid journals in the collection considered to be on a transformative path to full open access.

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"While there currently exists no fixed model of transformative agreement, many are being experimented with"

On the other hand, this model does not fully allay the fears of selective journals. Journals providing rigorous peer review with high rejection rates could argue that the Publish-and-Read model exerts pressure to publish a greater volume of research, potentially to the detriment of scientific quality. The agreement has also raised questions about global inequality. The DEAL consortium showed the bargaining power of a large alliance in a prosperous country to strike an economically favourable deal. However, researchers and librarians in the less wealthy Global South have understandable concerns that they could find themselves "locked out" of authorship – unable to negotiate acceptable terms for national licences and with their publishing opportunities then limited by high APCs.[4]

Other models have been trialled. SAGE and UNC-Chapel Hill recently signed a 1-year pilot deal, on the surface very similar to most Publish-and-Read agreements.[5] For the same price as a standard subscription, UNC-Chapel Hill authors will also receive publishing credits at a value of 50% of the deal's worth to fund open access publishing. Of course, these credits will not stretch to fund APCs for all articles published by the institution's authors, and difficulties may arise when it comes to deciding which authors are allocated credits. Additionally, for each paid APC, SAGE will give UNC-Chapel Hill credit worth half the value paid. This aspect of the model is inventive, though it does require a lot of communication between researchers and their funders and institutional libraries.

Authors who publish externally funded research will be obliged to pay an APC if their funding includes a publication budget, or if their funder offers a central budget to cover publication charges. Even when neither of these options apply, these authors are expected to directly request their funder to cover the cost. For each such paid APC, SAGE will give UNC-Chapel Hill credit worth half the value paid. While publishing should be viewed as an important stage of the research process, it remains to be seen whether funders who do not currently provide funds for APCs will be willing to shoulder further costs.

Overall, Projekt DEAL remains the most notable transformative deal struck. It will be interesting to assess the contract's impact on the publishing landscape once it expires in January 2022. Given the recent signing of an equivalent deal between Wiley and Jisc in the UK, it seems likely that the academic institutions responsible for the vast majority of research output will be tied into a global patchwork of similar transformative agreements. Going forward, it is probable that the consortia representing these institutions will push for reduced Publish-And-Read fees, especially as the "read" fee will become less valuable in an increasingly open access environment. Assuming publishers do not implement geowalling, researchers of institutions or nations not covered by these agreements may benefit from free research content, but at the same time could struggle to pay to publish their research in a world of uncapped APCs.[6] Somewhat ironically, smaller publishers may also suffer in this landscape, lacking the resources of their larger commercial rivals to negotiate such global licencing deals. Smits's parting description of Plan S as an icebreaker has rung true, but it remains to be seen whether, and if so how, full open access compliance will be pushed over the finishing line by January 2024.

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Ibuprofen and coronavirus

Back in March 2020, stories circulated about ibuprofen, a non-steroidal anti-inflammatory drug, and its effect on COVID-19. Dean Willis, Lecturer in Neuroscience, Physiology & Pharmacology at University College London, explains why inflammation is necessary for our bodies, and the effect ibuprofen might have for someone infected with COVID-19..

bit.ly/2X5Y6Sf

Tracking the spread and mutations of coronavirus

Physiological Society Member Thushan de Silva, Senior Clinical Lecturer at the University of Sheffield and Honorary Consultant Physician in Infectious Diseases is working on sequencing the genome of coronavirus and discusses why this is useful for tracking the spread and mutations of the virus. Continually sequencing the virus will be vital even once we have a vaccine, to ensure that it is effective against all strains.

bit.ly/3fKXJ7V

Designing scalable ventilators for COVID-19

Coronavirus patients sometimes develop a lung condition whereby the lungs can't provide the body with enough oxygen and need the help of a ventilator to stay alive. Physiological Society Member Federico Formenti, Lecturer in Human Physiology at King's College London tells us about a collaboration with the University of Oxford to produce ventilators, following the UK Government's call for support with design and production back in March 2020.

bit.ly/2WtEU1i

Using blood to treat COVID-19

Physiological Society Member Michael Joyner, anaesthesiologist and physiologist at the Mayo Clinic in Rochester, Minnesota is working on clinical trials using the blood of coronavirus survivors to treat patients. They are using the part of our blood that contains antibodies, called the blood plasma. As blood plasma is readily available, this approach could serve as a stopgap until drugs and vaccines are developed.

bit.ly/2T1dAWh

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Experimental design and irreproducibility in pre-clinical research

Michael FW Festing

Medical Research Council (MRC) Toxicology Unit, University of Leicester, UK

Too many pre-clinical experiments, mostly involving mice and rats, are producing results which cannot be repeated. This is probably because the scientists are not using statistically valid experimental designs. As a result, effects that are due to the research environment may be mistaken for treatment effects, leading to bias and irreproducible results. However, two designs, the completely randomised and the randomised block designs (likely familiar to physiologists working with farm animals) avoid such bias and have been used successfully for nearly a century in agricultural and industrial research and in clinical trials. In these two designs, subjects receiving the different treatments are randomly intermingled in the research environment, thereby avoiding environmental bias. Scientists engaged in pre-clinical research should be using these experimental designs.

Most scientists will now know that excessive numbers of pre-clinical experiments produce results that are irreproducible.[1] This leads to a waste of scientific resources[2] and excessive numbers of animals being subjected to unjustified pain and distress.

This note explains how scientists can design their pre-clinical experiments so as to maximise reproducibility by using the above designs. Some other rarer, named and statistically valid, designs are discussed in textbooks on experimental design but they are not discussed here (for more information see: Cox, 1958; Snedecor and Cochran, 1980).

The origin of randomised, controlled experiments

Randomised, controlled experiments were invented by RA Fisher when he was appointed as the statistician at Rothamsted Agricultural Experimental Station in the UK in the 1920s. His aim was to be able to detect small, but important, differences in the yield of different varieties of crops or following different fertiliser regimes.[5] He noted that there are two sources of such variation, which need to be controlled if unbiased and reproducible treatment effects are to be detected. First, there is inter-individual variability, controllable by choosing uniform subjects. In pre-clinical research this usually presents few problems because large numbers of high-quality, uniform animals, such as inbred and and pathogen-free strains of rats and mice, are readily available.

Second, there is the variability caused by the research environment. In an animal house, such variation could be associated with cage location within a room or position in a cage rack, variation in lighting levels, cage cleaning and the introduction of new bedding material. In shorter-term experiments circadian and other rhythms may add additional variation. Noise, including ultrasound not heard by the staff, may affect the animals, and the skill of those handling the animals and measuring the outcomes of an experiment may also vary over time. All these factors can cause extra inter-individual variation and need to be taken into account when planning powerful and unbiased experiments.

Two designs to use, one to avoid

Fisher developed two designs that provide some control of these sources of variation.

In the completely randomised (CR) design, the "experimental units" (either a single animal, or two animals in a cage counted as a single experimental unit) are numbered 1 to N (as in Fig. 1A). Then one of the treatments, *chosen at random*, is assigned to each subject (different shades in Fig.1A). This should be done in the office before the experiment is due to begin. Usually, equal sample sizes are used, although this is not essential.

Such randomisation is easily done using spreadsheet software such as Microsoft Excel. For example, if there are three treatments Lo, Hi, and Ctrl and a sample size of six, column A should have six "Lo", six "Hi", and six "Ctrl" entered into it. Column B should then have 18 random numbers generated by typing *=rand()* into cell B1. This can be replicated by pulling down on the bottom-right box. It will generate 18 random numbers. Columns A and B should then be selected and sorted on column B. The line number will then be the individual ID, and the assigned treatments Lo, Hi and Ctrl, in random order, will be shown in Column A.

The resulting list of subjects, each with one of the treatments assigned to it, is then taken down to the animal house and the subjects are given the appropriate treatment.

The result is a single set of subjects (experimental units), each receiving one of the treatments, determined at random. The subjects receiving the different treatments are randomly *intermingled* within the research environment, as shown in Fig. 1A. This is the design used in clinical trials because it can accept both the accumulation of patients over a period of time and unequal sample sizes. In pre-clinical studies it will normally be analysed using a one-way analysis of variance (ANOVA).

A randomised block (RB) design is shown in Fig.1B, again assuming that the experimental unit is a cage (housing either one individual, or two individuals with their results averaged for the cage). In this design, the experiment is split up into N independent groups or "blocks". So it is a "mini-experiment" with a sample size of one. For example, if there are three treatments, each block will consist of three cages, each receiving a different treatment, assigned at random.

The whole experiment will be made up of N blocks. So if the sample size is, say, six, there will be six blocks each consisting of three cages,each receiving a different treatment, or a total of 18 cages.

Treatments need to be assigned at random to each subject within each block. This can easily be done when setting up the individual blocks by writing the treatments on cards, shuffling them and displaying the order.

The individual blocks can be set up over any time period, to suit the investigator. For example, one block could be set up per day for N days. They don't need to be equally spaced. Spreading the work over a period of time by using the RB design could be useful if measuring the outcome is time-consuming or needs special apparatus.

The results from all the blocks are combined in the statistical analysis, which is a two-way ANOVA *without* interaction. The treatment means are averages of each treatment across all the blocks. The statistical analysis will indicate whether there are statistically significant treatment effects after removing the variation due to differences between the blocks. Such a two-way ANOVA should be readily available in all statistical packages.

The RB design cannot be used in clinical trials because it requires the ready availability of matched individuals. The one exception might be if identical twins were readily available.

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Then each twin would be assigned a different treatment and the pair of twins would represent a "block". This RB design, with just two treatments, is sometimes known as a "matched pairs" design.

The RB is the most widely used design in agricultural and industrial research because it provides better control of the environmental variation, so it is more powerful than the CR design.[6] One estimate, based on five RB experiments, was that a comparable CR experiment would need about 40% more animals to have the same power as an RB design.[7]

Another advantage of the RB design is that it allows the work to be spread over any time period (hours, days, weeks or months), to suit the investigator.[8] In this way, repeatability can also be built into it.

The RB design has already been used several thousand times, apparently without difficulty, in studying animal models of development. No litter of mice or rats is large enough to make up a whole experiment. So, each litter is regarded as a "block", with pups within the litter being the experimental units. Each pup receives one of the treatments. The results from the N blocks (litters) are combined in the statistical analysis, which is a two-way ANOVA without interaction.[9]

A third, but *statistically invalid*, design which might be called "randomisation to treatment group" is shown in Fig.1C. Scientists can often buy a group of animals that are all virtually identical. So they may see little point in randomising them.

However, as already noted, the research environment is not uniform. For example, if the scientist becomes more skilful as he or she measures the outcomes, this could lead to differences between groups that are only a reflection of this change in skill. So, in this design, variation in the research environment is *confounded* or mixed with any treatment effect, possibly leading to bias and false conclusions.

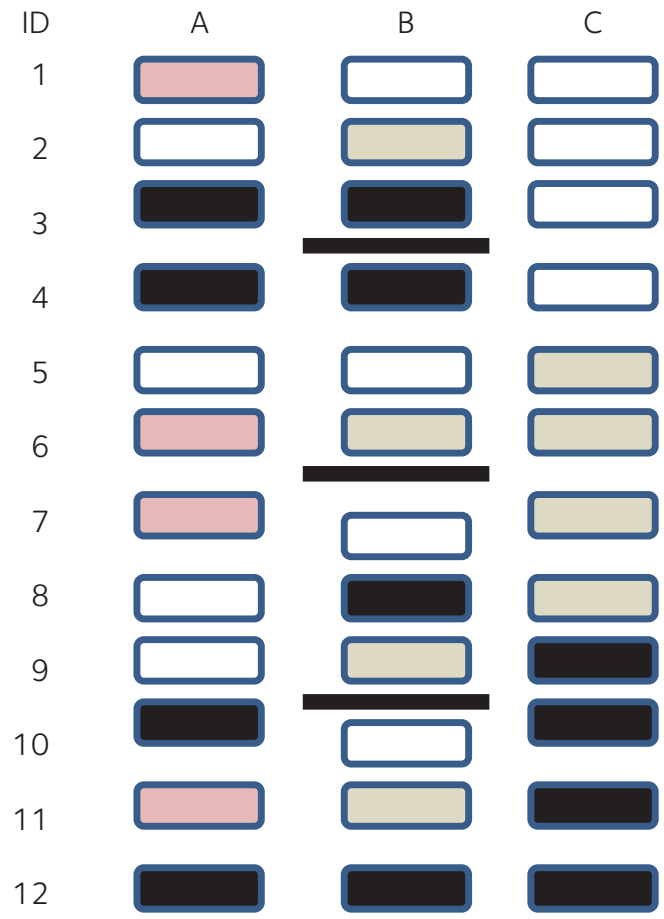

Figure 1. Three experimental designs, each having three treatments (shades) and a sample size of four.

A. The "Completely randomised" (CR) design.

B. The "Randomised block" (RB) design. Bars delineate the blocks.

C. The "Randomised to treatment". This is not a valid design.

Combatting irreproducibility

The "completely randomised" and the "Randomised block" are the only experimental designs suitable for widespread use in pre-clinical research. They both have "intermingled randomisation" in which subjects receiving different treatments are housed in randomised order within the research environment. This avoids bias, in which effects of the environment are mistaken for treatment effects. Further details on how to set up and use these designs are given elsewhere.[8]

The "Randomisation to treatment group" design (Fig.1C) is widely used in pre-clinical research. But *it is not statistically valid* because it doesn't randomise the *order* in which the experiment is done. As a result, it is susceptible to bias because environmental effects can be mistaken for treatment effects, as already explained. This can lead to false positive, irreproducible results. It may even be the main cause of irreproducibility in pre-clinical research.

The CR and RB designs have been used successfully for more than 70 years in agricultural and industrial experiments, and in clinical trials, without excessive levels of irreproducibility. They are the only statistically valid experimental designs suitable for widespread use in pre-clinical research. Scientists, the funding organisations, ethical review committees and journal editors should take note, and act accordingly.

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An interview with Greg Clark MP, new Chair of the Science and Technology Committee

Tom Addison

Policy Manager, The Physiological Society

Select Committees play an important role in both Houses of Parliament by reporting on and scrutinising areas of Government. In the House of Commons they are comprised of a minimum of 11 MPs and are able to call in ministers, officials and experts for questioning. Select Committees publish their findings in a report and the Governnment is expected to respond to any recommendations that are made. The Science and Technology Committee (S&TC) in the previous Parliament carried out inquiries into areas such as e-cigarettes, immigration policy for scientists, and the effectiveness of research and innovation spending.

Following the General Election in December last year, a new Parliament means new Chairs and Members for the House of Commons' 28 Select Committees. The Select Committees are responsible for scrutinising and reporting on the work of Government departments. The Physiological Society's Policy Manager, Tom Addison, met with the new Chair of the Commons' Science and Technology Committee, Greg Clark MP.

Greg has been the Member of Parliament for Tunbridge Wells since 2005 and has previously served in several ministerial roles including Secretary of State for Business, Energy and Industrial Strategy (BEIS), Minister for Universities, Science and Cities and Financial Secretary to the Treasury. During his time as Secretary of State for BEIS, Greg was responsible for the development of the UK Government's Industrial Strategy and establishing its four Grand Challenges including the Ageing Society target, which is the focus of The Physiological Society's report *Growing Older, Better*.

What are your priorities as Chair of the Committee?

This is an incredibly important and exciting time for science and technology. The changes and discoveries that are taking place and their rollout throughout the world, means that there are big questions for policymakers about how we can maximise the opportunities and impact while dealing with the regulatory questions that arise. So I really want to build up the influence of the Committee given the importance of the subject matter.

How does the science community contribute to policymaking?

The scientific community has a significant role to play in science policymaking. I am not one of those people who believes that we have had too much of experts! It would be paradoxical for my Committee not to be heavily influenced by the expertise that we are fortunate enough to have in this country. This happens through the written evidence the Committee receives and the oral evidence sessions we will hold. I would also hope, however, that I can deepen the existing informal links that I have developed. During my time as both Science Minister and Secretary of State [for Business, Energy and Industrial Strategy], I was lucky enough to engage with members of the scientific community, so this is an opportunity for me, and I hope other committee members, to be on pretty close terms with those that we need to be hearing from.

Can you explain the role of the Commons Science and Technology Committee and its relationship with its Lords' equivalent and the Parliamentary Office of Science and Technology (POST)?

The Science and Technology Committee is unique in that it doesn't monitor a single Government department. Science and technology recognise hardly any boundaries and the work of the Committee should be pretty free-ranging across all of the work of Government, the country and world at large. There is a strict procedural link to the Government Office for Science where the Committee is anchored [based itself within BEIS] but I think everyone would expect this to be interpreted quite broadly.

In terms of our relationship with the House of Lords, I think again uniquely, we have an equivalent committee, chaired by Lord Patel. I want to have a close relationship with the Lords' committee so we can be complimentary in terms of our inquiries.

We are very lucky to have POST [the Parliamentary Office of Science and Technology is the Parliament's in-house source of independent, balanced and accessible analysis of public policy issues related to science and technology], which adds to the work of the Commons' and Lords' libraries in giving specialist, expert and highly-respected research and analysis to help inform the work of the Committee and Parliament more generally.

As a former Secretary of State whose department included science, how does this help inform your new role as committee Chair?

Having been in Government, in a number of different posts, knowing how things are done in Government at every level supports the scrutiny function of my committee. I have also had some experience of appearing before select committees (including the one I now Chair!), so I may be wise to some of the attempts to avoid some of the difficult questions by some colleagues appearing in front of the committee!

More generally though, the scrutiny from Select Committees is something that I championed while in Government. Scrutinising Government is not only about challenge and due process, it is also about encouragement and shedding a spotlight on a shared endeavour – advancing and promoting science in our country and in our world.

How do you work with your Committee and how are topics for inquiries decided?

We are still waiting for the rest of the Committee to be appointed but it is worth remembering that I am its Chair, not the person responsible for dictating its agenda or content.

One of the things I want to do once the Committee is formed is to take the views of my colleagues on the Committee, all of whom will have the same motivation as I have – inquiring into the most important topics that relate to science and technology – to put together a work programme for the year ahead and beyond which is a combination of the most topical questions of the day but also more fundamental topics that require deeper, long-term analysis.

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How can physiologists get involved in your work on the Committee?

Physiologists and other life science researchers work in a particularly exciting time. Of all of the breakthroughs that are taking place, in terms of discovery and access, physiology and the life sciences more generally are replete with some of the country's best researchers.

In addition, these scientific discoveries bring with them important public policy questions, from how much public funding supports this research, the circumstances and conditions under which this funding is granted, through to the ethics and regulations of research and their experiments. I would hope that through The Physiological Society and its journals, there will be a pretty close dialogue between Members and all the members of my Committee, so that we are well informed and we can be asking the questions and giving the push in areas of interest to your membership.

How did you interact with Select Committees in previous roles?

My view of select committees was often seen as pretty subversive during my time in Government! Typically, the pattern in Government was to be rather resistant to the advice of Select Committees. In fact, where constructive recommendations were made, there were times that we would pretend we were already doing the things that were suggested! I always found this to be nonsensical. When you have a group of expert and motivated people like a Select Committee, taking evidence from people with even greater expertise, and coming up with recommendations and advice, you should fall on it with enthusiasm rather than try to resist it.

As such, during my time in various Government roles, I encouraged certain inquiries and always tried to adopt as many recommendations from Select Committees as possible as Government policy. I would hope that the ministers that appear before my committee might take the same view!

It is very important that any recommendations that come from the committee are well-informed, rigorous and evidence-based. That is foundational. I also think the strength of a Commons Select Committee is that it is drawn from elected parliamentarians of different political parties and different parts of the country. Public policy is not identical to scientific advice – the two need to come together to be effective and a good committee will help to bring together evidence-based expertise and deliverability that will make a difference to the people in the country that elect us.

How can the S&TC work to hold the Government to account over cross-departmental priorities and challenges?

The great thing about my committee is that by its nature, science and technology cross every department in Whitehall. For example, every department will have a Chief Scientific Adviser. It makes sense, therefore, for the committee to have a broad coverage.

Just as in academic life, some of the most interesting areas of inquiry are at the interstitials of disciplines. Silos are being busted apart in public policy, as in academia, so I have already had conversations with other committee Chairs, such as Environmental Audit [Philip Dunne MP] with common interests such as climate change, and the contribution of science and technology to tackling it, to try and scrutinise the whole of Government and that my committee will work closely and jointly to advance important causes.

What do you see as challenges to raising the profile of Science and Technology in Westminster?

One of the reasons I think the profile of science and technology, while reasonably high given the strengths we have in the UK, is that Parliament has been dominated by Brexit over the past 3 years. This has crowded out the problems that areas such as science and technology have. We have now formally left the European Union and while there are still negotiations about the future relationship to be concluded, in which scientists have an important stake, I hope this will be a time in which the potential, possibilities and innovations of the scientific community are a bigger topic of conversation in Parliament and beyond than perhaps they have been in the past 3 years.

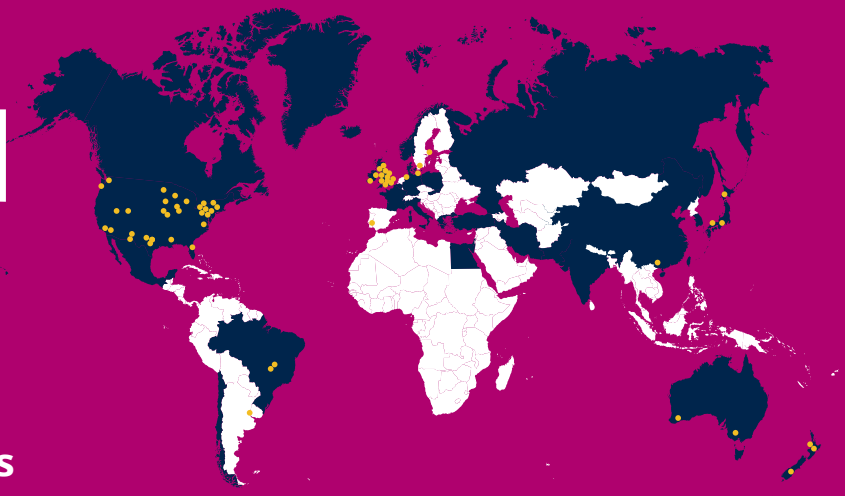
How can the innovations in physiology be translated into the evidence that is presented to the Committee and, by extension, Government policy?

It is really important that the S&TC should benefit from the best expertise, whether that comes from The Society or its journals, we need to be able to access the insights and breakthroughs that are being made so I am very keen that the Committee and its members, in taking evidence, have a close relationship with The Physiological Society, that we are well informed of what is breaking in the journals so that evidence can strengthen the recommendations we make for public policy.

https://doi.org/10.36866/pn.118.16

Reasons to submit to

Experimental Physiology



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An interview with John Cripps, our new external trustee

Julia Turan
Managing Editor, *Physiology News*

Where have you worked?

A mixture of investment banking, major firms such as Lazard and HSBC, and working directly in industry, companies such as Bass and Syngenta. The thread tying these together is a passion for helping clients or ideas grow, combined with a deep interest in science and what we consume.

What did you do?

In banking I principally worked in the Corporate Advisory business, understanding the needs of corporate clients of all sizes and then working with them to either solve a problem or plan a future way forward for the business or, in many cases, both.

The enlightening thing and great learning about working in industry at Bass and Syngenta was being able to not only help form plans but execute them, rather than moving on to the next client as one does in banking. However, there were downsides for my ego as, in my training month at Bass, the brewer, I was throwing barrels of beer off a Bass lorry early morning into a pub cellar in London when one of my old banking colleagues pulled up alongside me in his gleaming new Porsche, and just looked up and smiled!

What is your academic background?

Having studied mainly sciences before university I opted to read law for mainly family reasons. What struck me was the similarity in academic rigour and deep enquiry between the two disciplines; they both required immense hard work and dedication. In purely practical terms my law experience has been of great help in addressing the commercial aspects of innovation and science.

What attracted you to The Physiological Society?

Since my wife has a long-term complex medical condition, and her family included medics, we have been engaged at the sharp end of clinical medicine at leading hospitals for many years. This has taught us that, although there is huge value in the new biology disciplines, an overall understanding of a system remains just as important. Having all, or almost all, of the pieces of a picture puzzle is hugely valuable, but so is fitting them together and seeing the whole picture revealed.

What do you do when you aren't working?

Apart from occasionally losing a lot of golf balls I also enjoy helping my wife garden, albeit my habit of reclaiming what she calls spent plants and rehoming them does cause friction. I have a passion for plants and nature since I believe there are not many issues not solved in natural ecosystems somewhere on the planet. My other passion is being Warden of what I believe is one of the most beautiful shepherd's churches in England.

If you were marooned on a desert island what luxury would you take with you?

A mask, snorkel and flippers to enjoy the sea and what lies beneath.

STEM for Britain 2020

STEM for Britain is an annual opportunity to foster greater dialogue and engagement between early-stage researchers and Members. The 2020 iteration took place just ahead of the UK Government's decision to enforce a lockdown in response to the novel coronavirus in February.

As such, The Society was able to join over 400 researchers, politicians and those responsible for promoting science within Parliament to award the Physiology Prize for the second year. We asked Sarah Houston, STEM for Britain's Gold Award winner, and Egzona Morina, STEM for Britain Physiology prize winner, why they joined STEM for Britain and the impact that winning will have on their career.

Egzona Morina
University College London, UK

Before I properly started my PhD, I initiated a project called "BrainCamp Kosovo" that focuses on teaching high-school students in Kosovo about neuroscience. My institute, SWC, was more than happy to support it and so it has been running since 2018. I myself am from Kosovo and was lucky enough to have grown up in Belgium, a country that gave me many opportunities to get to where I am today. Therefore, I always look for opportunities to support and participate in any endeavour like STEM for Britain, where scientists/researchers come together and share their sheer curiosity for the world around us. It is precisely these endeavours/experiences that I want young students in Kosovo to experience and so I am also working hard in trying to organise conferences there. I believe winning this prize will definitely give me the confidence that maybe indeed I am the right person to take this task on and that while sometimes I have the PhD student blues, others recognise my hard work and acknowledge it.

Sarah Houston
University College London, UK

In 2017 I attended a Newton's Apple training event at UCL in which Stephen Benn and Stephen Metcalfe MP encouraged all students to apply for the STEM For Britain event for the opportunity to network with MPs and other scientists. I really liked the idea of making a poster that would be accessible but still impactful so I entered, and I was surprised and delighted to have won. Being awarded this prize has encouraged me to look further into how I, as a scientist, can work with MPs, policymakers and learned societies to ensure the Government are aware of the importance of scientific research in all aspects of our lives. I would encourage any early career researchers to apply for this event next year. It was a wonderful afternoon and the judges and other researchers were so friendly and willing to share their knowledge.

https://doi.org/10.36866/pn.118.19

Meeting Notes

The contribution of sport and exercise science to the Welsh economy: The Physiological Society hosts expert panel in Cardiff Bay

28 January 2020,
National Assembly of Wales, UK

Tom Addison

Policy Manager,
The Physiological Society

In January, The Physiological Society was delighted to hold an event on sport and exercise science (SES) in the National Assembly of Wales. Hosted by Assembly Members from across the Welsh political spectrum, we welcomed representatives from Welsh Government, policymakers, charities and sports club foundations to discuss the varied contribution of SES to the individual, public and financial health of Wales.

The event also marked the formal launch of The Physiological Society's Wales-specific labour market data that complemented its UK-wide report *Sport and Exercise Science Education: Impact on the UK Economy*. The report found that SES contributes to the economy not just in terms of increased tax revenue but also in terms of reduced burden on the health system and social services.

The Wales-specific factsheet noted that there is a huge opportunity for SES graduates. Across the UK, for every £1 that a student invests in their education in SES they will earn £5.50 in future wages. Graduates can expect to earn nearly £670,000 more over the course of their working life as a result of their SES education, compared with their peers that do not graduate from university.

Jobs for SES graduates are also geographically spread throughout Wales. Although Cardiff, Swansea and Newport made up just under half of the 2,440 new job postings for roles requiring an SES skillset between July 2018 and June 2019, both Wrexham and Rhyl had over 45 unique job postings. Nearly one in five SES graduates are employed in education or research, a welcome recognition of the contribution that SES graduates make to the research landscape of Wales.

Joining the panel's chair Dai Lloyd AM, himself a GP in Swansea and Chair of the Assembly's Health, Social Care and Sport Committee, were representatives from Public Health Wales, the Older People's Commissioner for Wales, Sport Wales, the University of South Wales and Bangor University. They discussed the value of SES for older people in Wales as well as the barriers to greater engagement among people from economically disadvantaged groups and people living with chronic conditions such as multiple sclerosis. We also heard about how SES graduates can make valuable contributions to the health of Wales in SES-related employment post-graduation.

Dafydd Elis-Thomas AM, Deputy Minister for Culture, Sport and Tourism gave the closing keynote. The Minister has been an AM since the Assembly's inception in 1999 and has previously served as its Llywydd (Presiding Officer). The Deputy Minister spoke about the extent to which Welsh tourism, and regeneration of towns that have previously been dependent on heavy industries, are being driven by sport and exercise innovations. For example, the former slate mine at Penrhyn has gone from being the world's largest slate quarry to being the home of the world's fastest zipline.

The event in Cardiff represents a fantastic template for future Society engagement with devolved parliaments. The next step will be to ensure that The Physiological Society continues to engage with the Welsh Government as we build future policy projects to address the challenges of an ageing society, as highlighted in *Growing Older, Better,* The Society's response to the UK Government's commitment to "five healthier, more independent years by 2035". Welsh research remains underfunded compared with other nations and English regions within the UK. By promoting the contribution of SES through events like these, we hope that governments at all levels will see the difference that physiology subdisciplines make to the lives of individuals both in terms of health and careers.

https://doi.org/10.36866/pn.118.20

Meeting Notes

Three Steps to Career Success: A Webinar Series for Early Career Researchers

February 2020

Eleanor Newton

Professional Development Officer, The Physiological Society

In February, we partnered with the British Society for Immunology to organise a three-part webinar series for early career researchers. This series supports the professional development needs of early career members by providing advice on building resilience, networking, and transitioning to independence. Participants could also learn that the challenges they face are shared by their peers. Over three lunchtime sessions, we asked participants to take a step back from their daily routines and ask their burning questions.

Building Resilience
John Tregoning and Cecilia Johansson, Imperial College London

"Research has highs and lows, often on the same day. We all need tools to help us cope."
– John Tregoning

John Tregoning and Cecilia Johansson highlighted difficulties that all researchers face, and strategies to help overcome them. They discussed obstacles such as rejected grant applications, failed experiments and bad supervisors. They suggested that the key is not to dwell on what has happened but to learn how to react, including celebrating small victories and planning next steps.

Building resilience isn't just about knowing how to manage difficult situations but also maintaining a healthy work–life balance, to recover quickly from difficulties. You can value being a scientist and still make time for life outside of the lab.

Members appreciated hearing these personal experiences and said that, "It is vital to know that people actually struggle in their careers. This is something that needs to be spoken about so we can deal with it better."

Another important lesson a Member gleaned was that, "Everyone has to find their own mechanism of building resilience. There is no universal path."

Networking
Dan Brayson, University College London

"Successful networking is critical to progressing in science; one conversation with the right person can change your career"
– Dan Brayson

Dan Brayson highlighted the importance of networking, and helped Members think about their networking goals and routes to achieving them.

Using examples, Dan identified some steps for identifying and pursuing networking opportunities. Members appreciated the advice on how to connect with potential collaborators or mentors, including how to maintain dialogues. Dan also recognised that whilst having a plan is important, it is also good to be open to spontaneity and suggestions from others.

The second part of the webinar focused on how an online presence can help achieve your networking aims. This centred around two key questions: who do you want to appeal to, and how do you want to come across? This helps determine what type of content or platform to use. Dan provided a specific example of how he communicated his research through video-blogging series "The Ultra Cycle Diaries", which he produced with The Physiological Society. Opportunities can arise by collaborating with your learned society. It can be as simple as just asking!

Transitioning to Independence
Viki Male, Imperial College London

Starting your own lab can feel daunting. But it's a chance to set your own research agenda – what could be more exciting than that?"
– Viki Male

Viki Male, Lecturer in Reproductive Immunology, aimed to help those preparing to transition to independence. As well as acknowledging the different routes to this (grants and fellowships), Viki reflected on her own experience in receiving a Sir Henry Dale Fellowship from the Wellcome Trust.

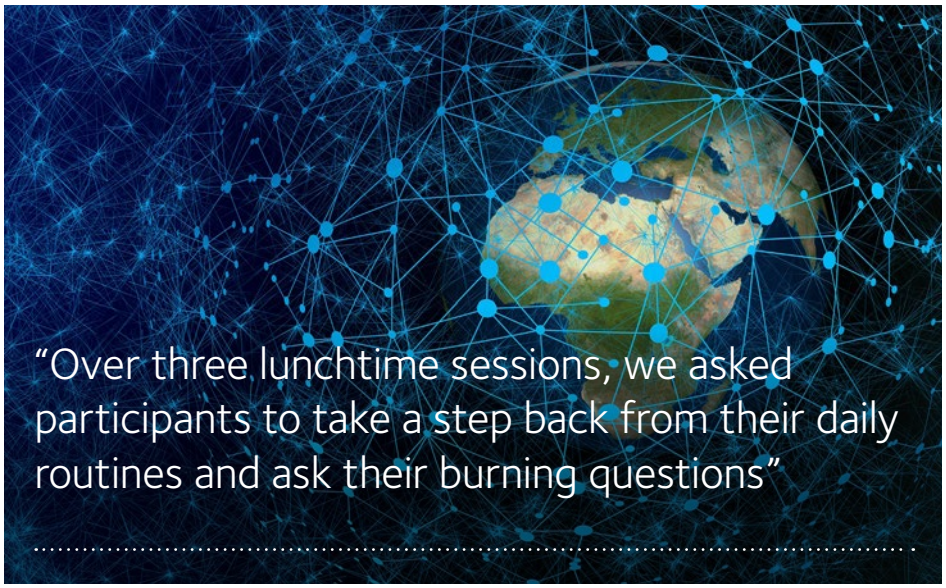
She broke down an application into three parts: person, project and place. As well as having a relevant publication record and reputation in the field, it is key to demonstrate how you can be independent, why the project proposal should be funded now (and not in 2 years), and why your chosen institution is required for the success of the project.

Viki also touched on how to continue career development once you have become independent. For example, organising seminars offers networking and collaboration opportunities.

This was a great chance for our Members to hear first-hand what it takes to start your own research group and sparked a variety of questions. It demonstrated how the pathway to independence is dependent on several factors such as personal circumstances, institution and long-term career goals.

Commenting on the webinar, one Member said: "I liked the way Viki highlighted the soft skills aspect of doing science research, which is often ignored as part of research training."

To request a link for webinar recordings, email **edufunding@physoc.org**



https://doi.org/10.36866/pn.118.21

Meeting Preview

Mental health in academia: Changing research culture

Week of 6 July 2020,
Future Physiology 2020

Francesco Tamagnini

University of Reading, UK

Join us for a workshop on mental health in academia at our first ever virtual conference, Future Physiology, during the week of 6 July 2020. Learn about the background and impetus for the workshop below:

I realised something was wrong during the second year of my PhD when I had my first panic attack followed by bouts of depression. In the first decade of my intellectual life, science filled me with a sense of wonder at the endless unknown, the feeling ancient Greeks called θαύμα (thauma). It gave me joy, so I decided that learning and doing science was my aim in life. In the second decade of my academic life, the pursuit of science was associated with disease, to the point that anxiety and depression became an important filter through which I viewed my intellectual and human experience.

Academic life, normally associated with human progress and intellectual challenge, is now also regarded as a possible cause of long-lasting mental disorders, both impairing the quality of life of academics and the reliability of their work.

While the causes of sporadic mental disorders are multifactorial and often hard to identify, a key precipitating factor for me was my PhD. I'm half-joking when I say my scientific motivation changed from thauma to trauma. My professional and personal development diverged during what should have been a key period of academic growth.

During the subsequent years of my career, I observed a similar pattern occurring in almost every PhD student I met. They came in fresh, enthusiastic and knowledgeable, and left broken. I could see how even their faces and bodies changed, becoming over- or underweight, looking older, more tired, and more detached. Unlike the portrait of Dorian Gray's, they were showing signs of decay directly on themselves.

Some of the thinking that can lead to people becoming mentally unwell may be as follows:

- Your success as a scientist is defined by the number of papers you publish and grants you're awarded. Your professional development (soft skills, technical skills. workshops) is often regarded as irrelevant at the workplace.
- You must keep your h-index high or you can't be successful.
- You must spend prolonged periods of time away from your family and friends because it is the only way to expand your network.
- It was your choice to do a PhD. It is a great experience and you should be grateful and positive. Mental health difficulties are "normal", everyone has them, and they should be regarded almost as part of your job experience.
- You have to stay at work long, but it is not clear how many more hours are enough.

In summary, a constant pressure towards performance rather than a person's intellectual development, generates a sense of frustration and imprisonment.

I have decided that this is stupid, unnecessary, and unfair. Something needs to be done. The good news is that people in academia are talking about this issue. Students, post-doctoral researchers, principal investigators, policymakers and funding bodies, are creating momentum around the conversation.

During this workshop, we want to discuss this topic, to promote the development of a healthier work ethic amongst scientists. The key questions will be:

- Are academics more prone to mental health disorders in comparison with the general population? If so, why?
- What steps can be taken to change academic culture to make it healthier?
- Would the improvement of scientists' mental health result in better science?

https://doi.org/10.36866/pn.118.22

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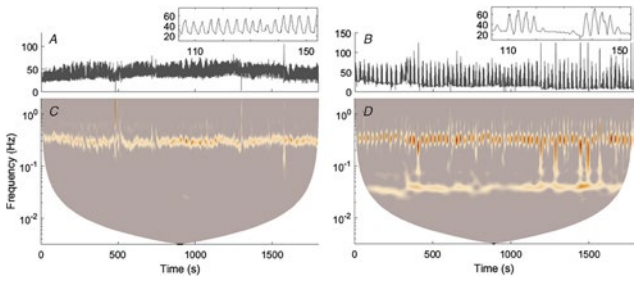
The Journal of Physiology

Relationship between cardiorespiratory phase coherence during hypoxia and genetic polymorphism in humans

Lancaster G et al. (20 January 2020)
https://doi.org/10.1113/JP278829

Amplitude modulation of respiration (periodic breathing) causes hypoxia and occurs in extreme conditions such as high altitude or in specific cardiovascular diseases. Periodic breathing is quantified using heart rate and breathing interactions but there is significant variability in these parameters during hypoxia. In this study, a new time–frequency analysis method was utilised to study periodic breathing in healthy individuals exposed to normoxia or hypoxia (normobaric and hypobaric). Periodic breathing was found to cause a regular oscillation, at a frequency below breathing, in subjects during sustained normobaric and hypobaric hypoxia. This novel time–frequency analysis technique showed an increase in cardiorespiratory wavelet phase coherence in hypoxia. The study further linked these physiological parameters to genetic polymorphisms, with NOTCH4 and CAT found to positively and negatively correlate with periodic breathing, respectively. This provides an interesting insight into the close association between genetic and physiological mechanisms and may explain the large variability seen in the measurement of periodic breathing in humans.

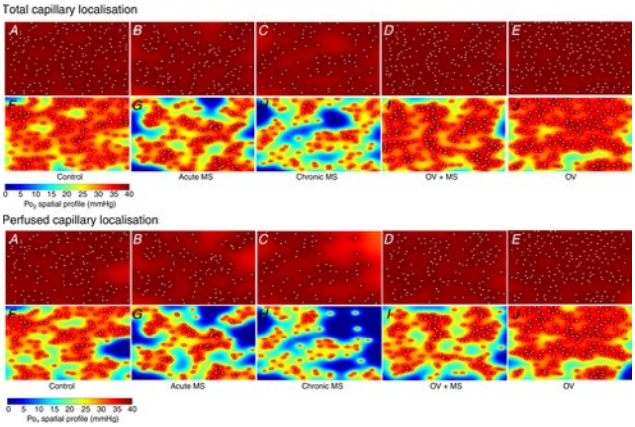


Continuous wavelet transforms of respiration signals during normoxia and normobaric hypoxia. *A*, breathing signal from one subject during normoxia. *B*, breathing signal from the same subject during sustained normobaric hypoxia (NH).

Impaired skeletal muscle performance as a consequence of random functional capillary rarefaction can be restored with overload-dependent angiogenesis

Tickle PG et al. (3 February 2020)
https://doi.org/10.1113/JP278975

During exercise, skeletal muscle relies on the microvascular system to supply oxygen and nutrients, and to remove metabolites. In settings of cardiovascular disease, such as heart failure, there is a reduction in capacity of the local microvasculature with lowered capillary density leading to local ischaemia and decreased capacity for exercise. In this study, polystyrene microspheres were injected into the extensor digitorum longus muscle in rats to block terminal arterioles and thus restrict blood flow to capillaries. Following acute microsphere injection (10 minutes), functional capillary rarefication was demonstrated, with reduced resistance to muscle fatigue. Over a 2-week period, as the vasculature began ischaemia-induced remodelling, through elevated angiogenesis, mechanical overload improved the perfused capillary density and fatigue resistance of the extensor digitorum longus muscle. These findings indicate that improving microvascular rarefication in skeletal muscle can improve resistance to muscle fatigue.



Simulation of muscle PO_2 at rest (A–E) and maximal rate of oxygen consumption (F–J) in representative images. Acute effects (B and G) are seen after injection of 200,000 microspheres. Model assumptions are provided in Methods. Areas of muscle hypoxia (highlighted in blue, where $PO_2 < 0.5$ mmHg) during exertion are increased after microvascular blockade (G and H) and this profile corresponds to an attenuated fatigue resistance.

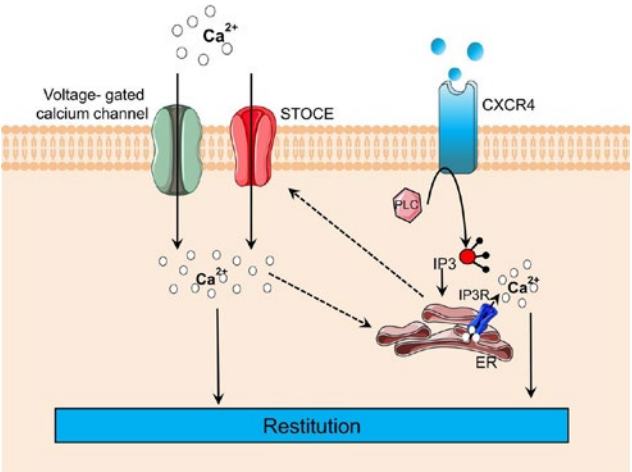
Physiological Reports

Multiple calcium sources are required for intracellular calcium mobilization during gastric organoid epithelial repair

Engevik KA et al. (8 March 2020)
https://doi.org/10.14814/phy2.14384

Raised calcium levels in gastric epithelial cells adjacent to damaged regions has been shown to accelerate gastric wound repair by increasing cellular migration and restitution of the epithelium. This study investigated the source of the raised calcium levels using gastric organoids with a genetically encoded calcium indicator, damaged by high-intensity light. The findings demonstrate that blocking entry of extracellular calcium through voltage-gated calcium channels or store-operated calcium channels on the cell membrane delays wound repair, as does inhibition of the phospholipase C/inositol triphosphate pathway, which releases calcium from endoplasmic reticulum stores.

https://doi.org/10.36866/pn.118.24

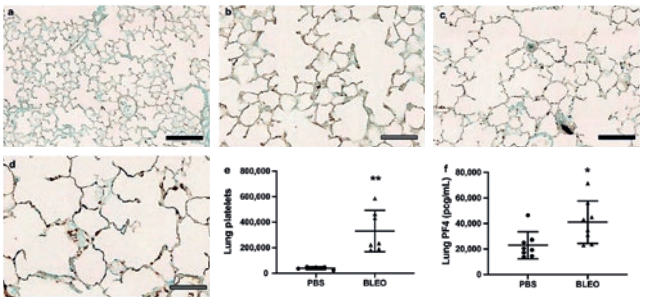


Schematic diagram of calcium mobilization during epithelial restitution. Dotted lines indicate more speculative pathways. $Ca2^{+}$, calcium; SOCE, store-operated calcium entry; PLC, phospholipase C; IP3, inositol trisphosphate; IP3R, inositol trisphosphate receptor

Platelet activation in experimental murine neonatal pulmonary hypertension

Halliday TM et al. (29 March 2019)
https://doi.org/10.14814/phy2.14386

Unlike diet-induced weight loss, bariatric surgery has been associated with changes in food preferences away from high-calorie choices and may also "reset" appetite-related hormonal responses. Such "resetting" may increase adherence to weight loss management protocols. Herein, the authors compared the influence of 10 kg weight loss by two different methods (Roux-en-Y Gastric Bypass [RYGB] vs. very low calorie diet [VLCD]-induced weight loss) on changes in appetite regulation and biomarkers of metabolic health. The VLCD consisted of the same calorie content and macronutrients provided to the RYGB group post-surgery. The authors found that weight loss was achieved more quickly in the RYGB group ($n = 6$) compared with the VLCD group ($n = 17$). Moreover, RYGB induced changes in appetite-related indices (including ghrelin, hunger, free fatty acids) that may be more favourable for sustained weight loss while VLCD induced changes that may promote weight regain.



Platelets are increased in the lungs of mice with bleomycin-induced PH. (a–d) Representative CD41 staining in 3-week-old mice treated with IP (a) PBS-20 × magnification, (b) PBS-40× magnification, (c) bleomycin-20× magnification, or (d) bleomycin-40× magnification, black filled scale bars = 100 μm, grey filled scale bars = 50 μm. (e) Interstitial platelets in neonatal mice following IP PBS or bleomycin treatment, $^{**}p < .005$ by unpaired *t* test, $n = 5$ PBS (4M,1F), $n = 7$ Bleo (5M, 2F). (f) PF4 levels in whole lung homogenates from neonatal mice following IP PBS or bleomycin treatment, $^{*}p < .05$ by unpaired *t* test, $n = 8$ PBS (6M, 2F), $n = 8$ Bleo (3M, 5F). PBS, phosphate-buffered saline; PF4, platelet factor 4; PH, pulmonary hypertension

EP Experimental Physiology

Effect of differential muscle activation patterns on muscle deoxygenation and microvascular haemoglobin regulation

Okushima D et al. (16 January 2020)
https://doi.org/10.1113/EP088322

As muscle work increases, so does the uptake of oxygen, but the rate of uptake varies in different muscles. The factors potentially facilitating this phenomenon are unclear but may include differences in recruitment, vascular control or metabolic control. Using different exercise protocols (knee extensor and cycling) while keeping track of muscle activation (measured by EMG), the current study found that both muscle deoxygenation and oxygen diffusive potential (measured by near infrared spectroscopy) depended on activation strategy in the *vastus lateralis* but not in the *rectus femoris*. Thus, both activation profiles and muscle architecture may play role in oxygen uptake from blood into muscle and account for differences observed between muscles despite same activity levels.

Exercise training attenuates angiotensin II-induced vasoconstriction in the aorta of normotensive but not hypertensive rats

Ramos de Oliveira P et al. (30 Jnuary 2020)
https://doi.org/10.1113/EP088139

The renin-angiotensin system mediates cardiovascular homeostasis and its dysregulation is associated with hypertension and other cardiovascular diseases. Exercise can also regulate vascular tone with changes in tone observed after an acute training stimulus and differences observed in trained compared sedentary individuals. This study investigated Angiotensin II responses in the aortas of trained or sedentary, hypertensive or normotensive rats. Hypertension was induced using the two-kidney-one-clip model. Thoracic aorta constriction was studied *ex vivo* in organ baths. Aortic constriction by Angiotensin II was blunted in the non-hypertensive, trained group compared to the corresponding sedentary group, in a manner that was dependent on endothelial-NO release. This blunting difference was not observed in the hypertensive groups, possibly due to oxidative stress. However, hypertension-induced medial layer thickening was attenuated in the trained group; thus training may confer some protection.

DOI: 10.36866/pn.118.26

Ecophysiology and climate change

Shaping our understanding of animal form and function

L Monica Trondrud

Norwegian University of Life Sciences, Norway

Alina L Evans

Inland Norway University of Applied Sciences, Norway

"Strange animals living in unusual situations have always fascinated humans" (Bennett, 1987). We are naturally drawn towards, and impressed by, animals with functional capacities that exceed our own. How do arctic mammals and birds withstand extreme cold? How can air-breathing animals stay under water for several hours? How can some birds cover thousands of kilometres in air without resting? And how do desert animals survive days, even months, without water?

These questions were first asked by curious biologists such as Laurence Irving (1895 – 1979), Per F Scholander (1905 – 1980) and Knut Schmidt-Nielsen (1915 – 2007), who dared to look beyond the laboratory and out in the wild. From their groundbreaking studies arose the field of ecological physiology, or simply ecophysiology. This field of biology concerns the relevance of the natural environment to an animal's physiology and evolutionary history, or to paraphrase Schmidt-Nielsen's aptly titled book, *How Animals Work* (1972).

All animals are adapted to their own environment with a particular range of ambient temperatures in which they can defend or manage their body temperature. Most animals also display other changes in physiology, morphology and behaviour in response to the natural fluctuations in their environment. The major drivers of these changes are energy availability and utilisation (i.e. energy balance or homeostasis), the need for thermoregulation and water balance.

For example, many arctic mammals adapt to the bitter cold by having thick insulative fur that conserves body heat, by going into torpor or hibernation, the periodic lowering of body temperature and metabolic rate, or by migrating to warmer climates. Desert mammals avoid overheating from the scorching desert sun by having thin fur and large evaporative surfaces, and often are behaviourally adapted by avoiding being active during the midday heat. Some desert animals have extraordinary renal adaptations that even allow them to forego drinking water.

Many ungulates in temperate and arctic zones show remarkable adjustments in heart rate, body temperature and activity levels in response to seasonal changes in ambient temperature and food availability. The Svalbard reindeer (*Rangifer tarandus platyrhynchus*, Fig. 1), for example, has the most extreme variation in resting heart rate between winter and summer known for any ungulate to date (Arnold *et al.*, 2018). Arctic and temperate rodents are faced with similar constraints but cope by going into hibernation, or torpor, and lowering their body temperature and subsequently their energetic demands. A remarkable example is the Arctic ground squirrel (*Urocitellus parryii*), which allows its core body temperature to drop below 0°C during torpor bouts lasting for several weeks. During torpor, their metabolic rates drop to 1–2% of basal metabolism, and every 10–21 days they spontaneously

https://doi.org/10.36866/pn.118.26

Figure 1. A Svalbard reindeer has short legs, short nose and small body compared with other reindeer subspecies. It also has the thickest fur of all reindeer subspecies, allowing it to withstand the extreme colds in the High Arctic winters.

rewarm to euthermic levels (36 – 37°C) for about a day before re-entering torpor (Williams *et al.,* 2016), a physiological feat that even other hibernators would only dream of achieving.

Some birds avoid the food-depleted and cold Arctic winters by migrating. Long-distance flight is energetically costly and is also physiologically remarkable. The 100 g Arctic tern (*Sterna paradisaea,* Fig. 2), for example, travels an astonishing 80,000 km each year to breed in the High Arctic, and overwinter in the Antarctic. Unique adaptations, including specialised lungs, allow birds to fly for such long periods of time (Egevang *et al.,* 2010).

Ecophysiology and technological advances

Since the rise of ecophysiology, advances in technology have continuously opened up a new world of possibilities for physiological studies. It's been 80 years since Scholander first described the three stages of diving (apnoea, bradycardia and peripheral vasoconstriction) in seals during forced dives in a bathtub, and 64 years since Gerald L Kooyman developed the time–depth recorder that was used to record the depths of voluntary dives by seals (Kooyman, 1966). Many years later, the first heart rate recorder was used during seal dives (Hill *et al.,* 1987), and today, almost any aspect of physiology and behaviour can be studied with bio-logging devices. Indeed, these have been used in a range of taxa including reptiles, birds, fish and mammals. With the advance in technology, the possibilities within ecophysiology have expanded tremendously and bio-logging devices allow us to understand the intricate details in the timing and coordination of physiological events without the stress and physiological, behavioural and ecological alterations associated with keeping animals in captivity during the recordings. This has given us new insights into long-distance migration in birds, diving physiology and thermoregulation in bears during hibernation, to mention a few.

"Ecophysiology … concerns the relevance of the natural environment to an animal's physiology and evolutionary history"

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Ecophysiology in the field

The above-mentioned topics are particularly difficult to study without the use of bio-logging devices as they occur in situations that are "beyond" our reach. We will exemplify this by highlighting some key processes occurring before and during hibernation in brown bears (*Ursus arctos*, Fig. 3*)* studied by Evans *et al.,* 2016. In that study, in free-ranging bears, they measured body temperature (T_b), heart rate, heart rate variability as a proxy for autonomic nervous system (ANS) levels and locomotor activity over 5 years. They found that brown bears prepare for hibernation by reducing activity levels, heart rates and T_b several weeks before entering the den. Denning appeared to be tightly coupled with metabolic suppression and, unexpectedly, T_b rose 2 months before den exit and was driven by ambient temperature (T_A), not by autonomic nervous system activity, which only began increasing 3 weeks before den exit. This indicates that passive rewarming occurred before the SNS began to restore euthermic metabolism in the last 3 weeks. It was not until the ambient temperatures reached the bear's lower critical temperature that bear exited the den, indicating that perhaps the bear exits when the den becomes "too hot". Ambient temperatures seem therefore to have a major impact on den exit in brown bears. With rising global temperatures, this dependence may cause shifts in the timing of den exit and potentially lead to mismatches in food availability (Evans *et al.,* 2016). The authors tried to measure den temperature and to use camera traps to document den exit. Unfortunately, the bears turned out to be very sensitive to disturbance and most changed dens after captures.

Working with wild animals often requires an immense amount of equipment, logistics and planning. For example, it takes a minimum of five people, three snowmobiles and one custom-made fish net to catch a Svalbard reindeer: two drive the catching snowmobiles, two people hold the net between the two snowmobiles, and the last person pulls the sled with handling equipment, polar bear protection (rifles), lunch and most importantly, hot blackcurrant juice. Reindeer don't look like fast runners, but they may surprise you. We often had sore legs and bruises from holding onto the snowmobile like bull riders, as the driver took a U-turn uphill chasing the reindeer. It is not normal, and probably unpleasant, for a reindeer to be chased, captured and handled by humans, so we try to minimise the number of captures, marking and recapture, and optimise monitoring equipment to track individuals throughout the year. Since 2009, we have been using GPS collars and activity recorders to track their movement throughout the year, and some lucky individuals have been equipped with heart rate and temperature recorders (Fig. 4).

Before the first winter captures of brown bears, we started calling black bear researchers to gain insights from their experiences. Black bears are often captured in the dens, but nobody had dared to try this with brown bears. To minimise our risks we decided to start with smaller bears, who were old enough to not be hibernating with their mothers (who would be much larger and aggressive in the protection of their young). To our surprise, unlike the black bears, some of the brown bears heard us coming and were wide awake! This, along with the extreme cold, presented significant challenges, especially when you found your ultrasound batteries dead, or worse when you found one of those bears awake and your tranquiliser solutions were frozen in the darts!

"This, along with the extreme cold, presented significant challenges, especially when you found your ultrasound batteries dead, or worse when you found one of those bears awake and your tranquiliser solutions were frozen in the darts!"

Similar to the Svalbard reindeer studies, we were dependent on snowmobiles to carry the people and the gear. We have four local forest rangers who volunteer with their snowmobiles to transport people and gear, and who also participate in the captures. After each bear capture, one snowmobile machine rushes the first samples back to the cars, and then we have a rally driver who is also a biomedical technician who races the samples back to the fieldstation and begins preparing them at room temperature. After the captures are completed, there is a mountain of logistical work to ship samples on dry ice across Europe but now, some years later, nearly 30 European researchers are able to study samples from these hibernating bears.

Consequences of climate change and physiological constraints

One of the consequences of climate change is the increasing frequency of extreme weather events such as heat waves, drought, floods, hurricanes, and winter warm spells in the Arctic (IPCC, 2013). In addition to immediate mortality from heat stress, dehydration or drowning during extreme weather events, habitat loss and reduced food availability may have consequences for reproduction and growth in the future. In the Arctic, winter warm spells and "rain-on-snow" events are occurring more frequently now than before, resulting in thick layers of ground ice, which "locks" in food and makes it inaccessible to herbivores. While most Arctic mammals build up internal fat stores that serve as a buffer against starvation in winter, these stores are not sufficient for non-hibernators to survive throughout the winter without foraging (Blix, 2016). Severe "rain-on-snow" events have in some instances led to population crashes in Arctic birds, rodents, and ungulates. Contrastingly, warmer summers may facilitate increased fat storage in the autumn, increasing the chance of survival over winter (Albon *et al.*, 2017). Studying the energetics of animals in these changing environments can help us understand the impacts of climate change on their coping capacities.

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Figure 2. The Arctic tern migrates up to 80,000 km each year, spending summers in both the Arctic and Antarctic. Here an individual is incubating on its ground nest in the High-Arctic Archipelago of Svalbard.

Figure 3. A brown bear is put into the den after being captured for ecological research (Evans *et al.*, 2016).

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Figure 4. Alina Evans performing surgery on a sedated Brown bear. Here she is implanting a subcutaneous heart rate logger that allows them to investigate heart rate during hibernation in the bears. Photo credit: Ole Frobert. This image is published under CC-BY-SA 4.0.

This may be particularly important for species in vulnerable areas such as the Arctic.

Global warming is leading to the gradual increase in the Earth's surface temperature. For arid-zone birds, direct effects of increasing temperatures can range from extreme heat waves causing lethal hyperthermia or dehydration, to chronic exposures to sublethal temperatures over several weeks. For birds inhabiting southern Africa's Kalahari Desert, one study predicted that the risk of chronic exposure to sustained hot weather (sublethal temperatures) can lead to progressive loss of body condition, delayed fledgling and breeding failure (Conradie *et al.*, 2019). When temperatures reach a certain threshold, the birds fail to thermoregulate while foraging at the same time. The foraging trade-off results in diurnal mass loss instead of mass gain, which over time leads to overall reduced fitness. By the end of this century, the authors predict that much of the Kalahari's avian biodiversity will be lost.

Concluding remarks

Ecophysiology is an essential part of understanding how animal form and function shape the behaviour and population dynamics of animals. For long-lived species, evolutionary adaptations do not occur at the same rate as their climate changes. As climate change progresses, the need to understand animal responses and adaptations to change is essential. Ecophysiology is facilitating the research on animals' capacity to respond to change and the mechanisms behind these responses.

We conclude this piece by promoting our virtual online conference Future Physiology 2020 titled "*Physiology in a changing climate: the interdependence between physiology, behaviour and the environment*." During this conference, we will explore how we can incorporate thermal and metabolic physiology into forecasts of future populations of both humans and animals. Visit our website to register: **physoc.org/events/future-physiology-2020/**

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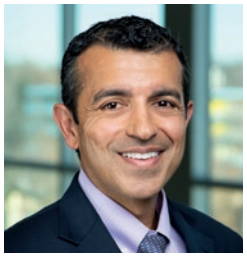
Sex in studies

The first level of personalisation

Natasha A Karp

AstraZeneca, Cambridge, UK



Rohit Katial

AstraZeneca, DE, USA

Karen Thacker

AstraZeneca, Cambridge, UK

Precision medicine is touted as the future; the approach to improve therapeutic outcomes by putting our patient at the centre of the healthcare system. Our sex is a fundamental evolutionarily conserved feature that divides our population into two. Yet our bench-to-bedside research neglects sex differences and assumes that knowledge gained from studying males will translate. Sex differences in disease prevalence, symptoms and progression, together with differences in treatment efficacy and side effects, are well accepted (Yoon, *et al.*, 2014). Examples are being published that suggest the evidence base of medicine may be fundamentally flawed. We need to strive for the inclusion of both sexes and then explore the data assessing for a sex difference to ensure our research is representative of the target population. Embracing this change should improve translation and efficiency of science.

Current state of play

Twenty-seven years ago, the NIH Revitalisation Act mandated the inclusion of women in clinical trials. However, women remain under-represented and are rarely included in the early phases of clinical trials (Feldman *et al.*, 2019). Exclusions are more likely in early testing with phase 1 and 2a trials more affected than phase 2b or 3. This risks drugs that might be more effective or safer in women being dropped from early clinical development due to poor efficacy or safety concerns in males.

In non-clinical research, researchers have highlighted that we predominantly study only one sex, typically males (Beery and Zucker, 2011, Fig. 1) and this bias has not changed in over 20 years (Mazure and Jones, 2015). The bias is apparent, even when the disease of interest is a female-prevalent disorder (Yoon *et al.*, 2014).

The risk of studying only one sex has been highlighted recently in the study of pain. Scientists at McGill University in Montreal, Canada decided to not follow the convention of their community and instead studied both sexes. They concluded that the reaction to pain was highly dependent on the sex of the animal and whilst the symptoms of pain might look similar the pathway mediating the pain could be highly distinct (Sorge *et al.*, 2015). Regulators, funders and publishers are pushing, across all stages of the research pipeline, to study both sexes. Whilst some progress is being made in the clinical research arena, non-clinical research is lagging behind. Most non-clinical studies, whether *in vivo* or *in vitro*, are conducted on only one sex, typically male. Whilst in clinical trials, inclusion of women is poor and analysis frequently fails to consider sex as a potential variable.

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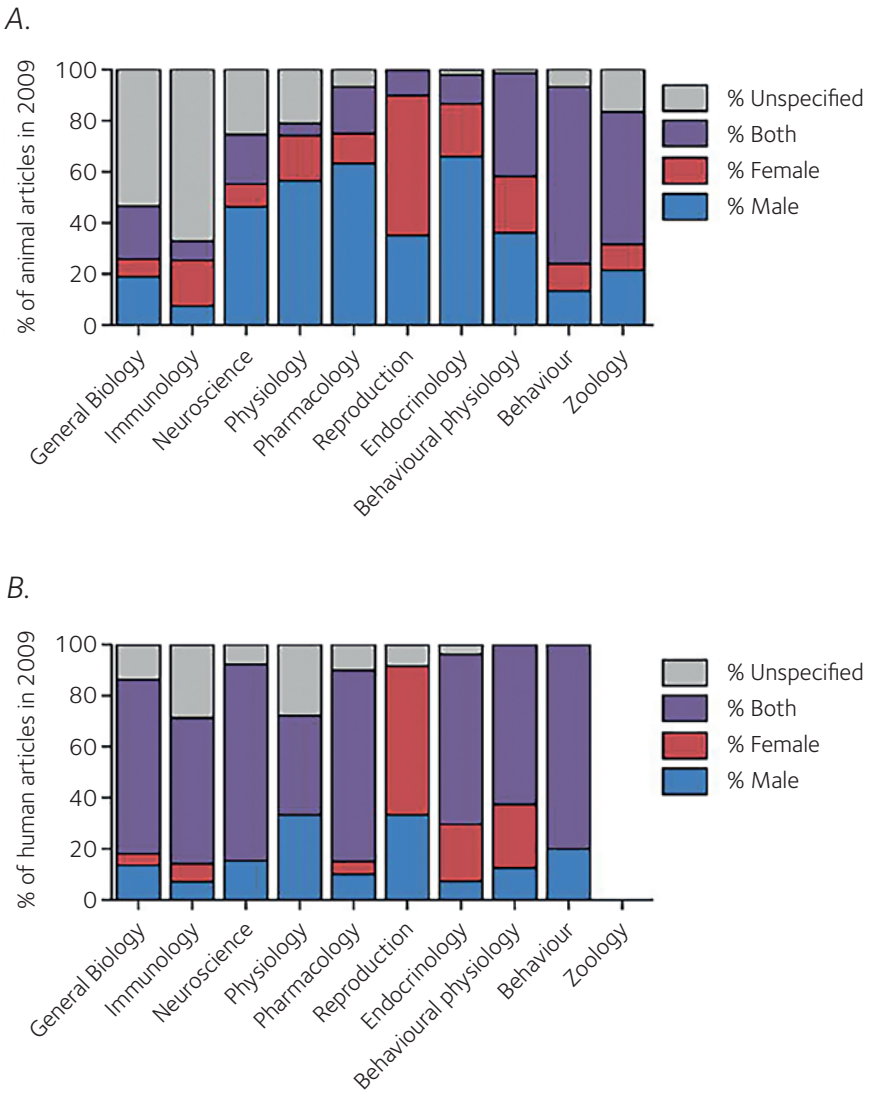

Figure 1. Distribution of studies by sex and field in 2009. *A.* Percent of articles describing non-human animal research that used male subjects, female subjects, both male and female subjects, or did not specify the sex of the subjects. *B.* Percent of articles describing human research in the same categories. The zoology category was excluded because of insufficient use of human subjects in this field to form an accurate estimate. Reproduced with permission from Beery & Zucker, 2011. © 2010 Elsevier Ltd. All rights reserved.

"In clinical trials, in spite of the requirement to include women, women are under-represented and frequently omitted from trials"

A paradigm shift through regulators, funders and journals forcing change

The historic focus on male subjects in clinical studies arose from risk management. In the 1970s, the priority was preventing adverse events on unborn foetuses, who had not consented to the clinical trial, and effectively banned the inclusion of women in clinical trials (Liu and Dipietro Mager, 2016). This was understandable in the wake of thalidomide where ten thousand children were born with malformations, at a time when oral contraceptives were new, their reliability uncertain and potential drug–drug interactions (DDIs) poorly understood. This position was reversed in 1993 with the NIH Revitalisation Act. Since then, regulators have instituted various policies to ensure proper inclusion of women in clinical trials. For example, the FDA has issued over 19 guidance documents and reports on the various aspects of inclusion of women in clinical research. Originally, women were only included once all relevant safety testing was complete. Now, the prerequisite non-clinical data requirements increase with the duration of the study and number of women included. It is often possible to enrol women in the very earliest single-dose studies with no more information than is required for men. The commonly used terminology of 'first time in man' may be misleading drug developers to believe that women should not be included until safety in men has been established. In reality, women can be included from the earliest clinical trials, using effective contraception (Clinical Trial Facilitation Group, 2014), when knowledge of the mechanism of action of the agent and the type of pharmaceutical agent are understood and the clinical trial meets criteria such as limiting time period of exposure and limiting the number of women exposed (ICH Harmonised Tripartite Guideline, 2009).

In pre-clinical research, multiple funding agencies, journals, and professional bodies have initiated programmes to encourage us to include both sexes in our research. As these advisory statements and initiatives had little impact, funders (such as the National Institutes of Health or Swedish Research Council) took the unusual step to mandate change and require consideration of sex in all applications from 2016. Likewise, journals such as the *British Journal of Pharmacology* recommend both sexes are studied and if not, a rationale must be included in the manuscript as to why it is not relevant to the experimental question (Docherty *et al.*, 2019).

Why do we often study one sex?

The scientific method uses experiments to simplify a complex world to generate a testing space where we can isolate cause and effect. We then generalise the results.

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"Failure to consider sex in our experiments could lead to missed opportunities and erroneous conclusions"

The level of complexity in biology is such that we have to make hard choices when we design experiments to generate "doable problems" allowing us to incrementally unravel the biological story. Historically, we have assumed sex isn't a big deal, but this is now being questioned.

For *in vitro* research, the predominant reason for only studying one sex appears to be cultural; where most scientists perceive the sex of the cells is irrelevant (Shah *et al.*, 2013). This position arises if the differences between the sexes is believed to arise from hormonal exposure. Recent research highlighting large scale differences in gene expression between the sexes has led to the theory that the underlying evolution for sex differences often works at the level of gene expression (Mealey, 2000). This is supported by the observation that sex differences exist between cells before the onset of hormonal exposure (Shah *et al.*, 2013). Failure to consider sex in our experiments could lead to missed opportunities and erroneous conclusions. Consider the following example: researchers looking at the potential of muscle-derived stem cells to efficiently regenerate skeletal muscle in mouse models for muscular dystrophy found significant heterogeneity in the response (Deasy, 2007). Instead of walking away from the research, they explored the heterogeneity and found that the cell sex is a variable that considerably influences the regeneration abilities of the stem cells.

For *in vivo* research, the 3R (Replace, Reduce and Refine) ethical framework, has also encouraged the study of one sex. Historically, the Reduce element has been interpreted as a requirement to focus on minimising the absolute number of animals within a single experiment and thus encouraging us to study in a narrow testing space and to generalise the results. This approach ignores the fact that the production of male animals involves the production of female animals and there is a welfare burden from this under-utilised outcome from breeding. Males were then selected because it was believed that the oestrogen cycle resulted in female animals being more variable and consequently a higher number of animals would be needed within an experiment to detect the same size effect. A meta-analysis has highlighted this is a flawed belief, but the legacy remains (Prendergast *et al.*, 2014, Fig. 2).

Practically, can we study both sexes?

In *in vitro* research, including sex as a biological variable can be challenging. Firstly, the sex is often not known for established cell lines as the chromosomes can become unstable. Secondly, many of these studies use immortalised lines generated from one individual. We cannot determine whether the differences would be due to a sex effect or individual differences. The critical step here is to understand the limitation of these studies and report the sex of cells when known. The increasing use of primary cells will allow studies to encompass sex as a biological variable going forward.

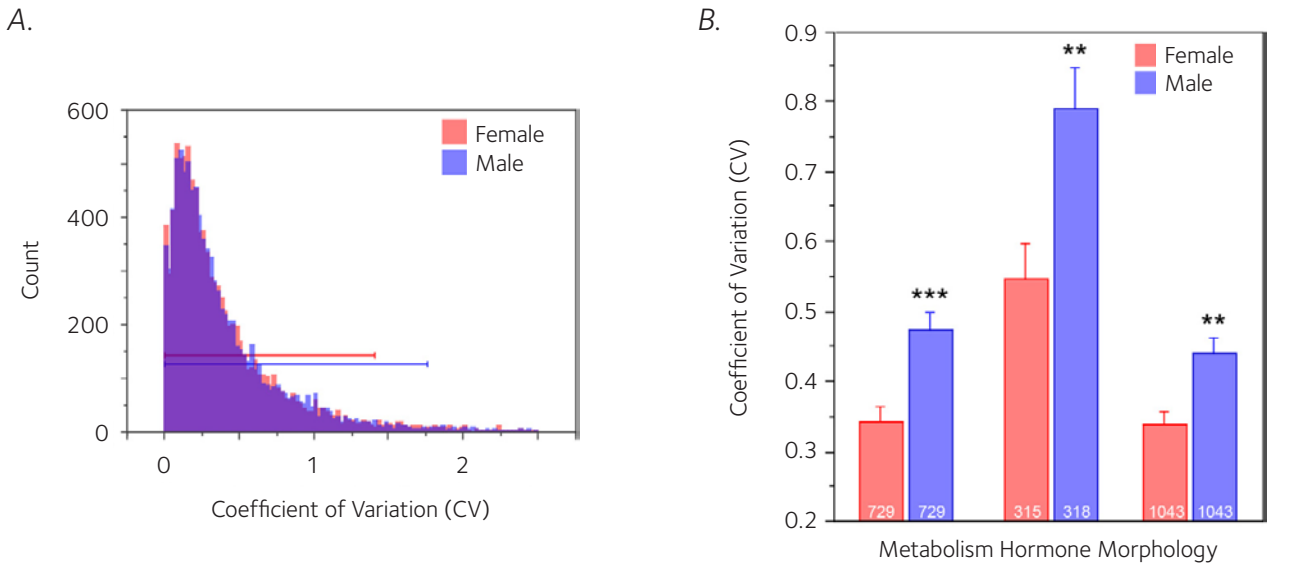

Figure 2. Mean trait variability is no greater in female than male mice. *A.* Coefficients of variation (CV; S.D./mean) for phenotypic traits (n = 9932) of male (blue) and female (red) wild-type mice as reported in 293 peer-reviewed articles published between 2009 and 2012. Purple shading indicates overlapping areas of the blue and red histograms. Horizontal brackets encompass 95% of the data points for each sex. Histograms do not depict outlier CV values (CV > 2.5), which represented a minority of the total data points (male mice: n = 338 [3.4% of total data]; female mice: n = 247 [2.5% of total data]). *B.* Mean + S.E.M. CV values for trait categories in which male CVs significantly exceeded female CVs. Numbers of CV values contributing to each mean are indicated along the abscissa. $^{**}P < 0.005$, $^{***}P < 0.001$ vs. female value.

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In *in vivo* research, there is resistance from misconceptions and practical challenges that hinder the use of both sexes. The most common argument against the use of both sexes is the misconception that you would need to double the sample size. In fact, it is recommended that you maintain your original design but split the animals for a treatment between the two sexes (McCarthy, 2015). The sensitivity is maintained because you now have a factorial design and the treatment effect is estimated from both sexes simultaneously (Karp and Reavey, 2019). This approach does mean these experiments are not powered to assess whether the treatment effect depends on sex, but will allow us to see a large difference if it exists. Studying both sexes does introduce additional practical challenges to ensure the process avoids the introduction of systematic bias and the experiments appropriately manage the welfare needs of the animals. For example, exposing a rodent to scents from unfamiliar animals or the opposite sex can lead to anxiety in the animal or changes in the experiment measure (Hurst, 2005). Different labs approach this challenge in different ways, some through cleaning whilst others accept the complex scent environment. We need to share our experiences and the strategies we develop so that we can learn how to manage this new paradigm.

In clinical trials, in spite of the requirement to include women, women are under-represented and frequently omitted from trials. Despite the change in regulator position, advancement of reliable contraceptives, improved DDI evaluation (Stewart *et al.*, 2016) and greater knowledge of drug targets in embryo-foetal development, this stance has been slow to change (Liu and Dipietro Mager, 2016). The question should be not whether we should include female subjects but why shouldn't we. To enable this, we need to ensure the criteria for inclusion of women in the first-in-human clinical trials are widely understood and risk assessments are based on literature and early non-clinical data when possible. Many phase I studies have a duration of less than 14 days and therefore can include small numbers of women of childbearing potential without any additional testing. Most phase II trials can include women by conducting preliminary embryo-foetal development studies earlier in drug development programmes (ICH Harmonised Tripartite Guideline, 2009). These steps combined with an inclusive approach to clinical study design will result in programmes that will better serve their patient demographic. In addition, these changes will increase the pool of volunteers/patients eligible for inclusion in clinical trials, which will speed up recruitment. This will help bring safe and effective medicines to the market more quickly, which is in the best interests of both patients and drug developers.

Driving change

Studying both sexes is essential and will improve experimental efficiency and enable social equality where our research pipeline represents the intended community. For over 20 years this issue has been discussed; however, sex bias is embedded in our research pipelines and culture. We don't always report the sex, we often only collect data on one sex and we frequently ignore sex in the analysis when we have collected both sexes (Karp and Reavey, 2018). Change is hard. The tide appears to be turning; we are moving from recommendations to requirements. Within each of our domains, we need to provide the leadership to build coalitions that build a vision and support change. These coalitions should then consult to identify and find solutions to the blockers that are resisting the change and implement strategies to strengthen the driving forces for change (Karp and Reavey, 2018). The issues are multifaceted and the solutions to support this change need to be bespoke for your community. The question is, are you going to start this journey and embrace sex as a biological variable?

Natasha Karp, Rohit Katial and Karen Thacker are employees of AstraZeneca and own shares in the company. We have no conflicts of interest with the subject matter or materials discussed in this manuscript to declare.

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Time to win gold

Could chronobiology make the difference between winning and losing for elite athletes?

Luis Mascaro

Monash University, Australia

Elise R Facer-Childs

Monash University, Australia

Timing is everything. It allows us to anticipate the best time to conserve energy, identify the time of peak performance, recognise when attention may be sub-optimal, and find the most effective time for nutritional intake. The study of timing and cyclic phenomena in humans is termed human chronobiology (*chrono* = time) and has many implications for the elite athlete.

Circadian timing and athletic performance

Circadian biology is a subset of chronobiology that involves the study of cyclic phenomena with a period of approximately 24 hours (*circa dian* = about a day). The circadian system, commonly referred to as our internal clock, is responsible for temporally organising our physiology across the 24-hour day. This includes setting the timing of gene expression in individual cells to regulate cellular metabolism and growth/repair, to coordinate the ~24-hour rhythmicity in cross-tissue processes such as hormone signalling and core body temperature. It is perhaps unsurprising then, that there is a daily rhythm in athletic performance.

Such internal timing has a bidirectional interaction with timing of behaviours such as when we eat, sleep, and work. Our physiology can affect our behavioural choices and our behavioural choices can determine the timing of exposures to which our circadian system aligns; the best studied example is the light–dark cycle. For many individuals, their diurnal (daily) timing of behaviours will be strikingly different on work days compared with free days such as weekends as a result of behavioural preferences. Other social commitments such as having to get up earlier to get kids ready for school could also affect the exposures to which the circadian system aligns and be at odds with behavioural preference.

Information on timing of actual behaviour, timing of behavioural preferences, and timing of circadian rhythms (for instance, whether they peak or trough earlier or later in the day), in whole or in part, can be used to classify individuals into different clock 'types'. Being an early, intermediate or late type is known as your circadian phenotype (sometimes referred to as chronotype) (Facer-Childs *et al.*, 2020). An early type will tend to rise earlier, perform at their best earlier, and go to sleep earlier. The opposite is true for a late type.

Clearly, given the multitude of physiological processes that are circadian regulated, the synchrony between our internal clock time, behavioural preferences, and actual timing of activities (external time), athletic performance can be affected. Not only will chronobiology affect the time of day of peak performance but also the ability to achieve maximum possible performance levels more generally.

This is very important for the elite athlete. Being able to manipulate peak timing of performance, for instance, could result in a significant competitive advantage. As an

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"One's circadian phenotype is on a spectrum, with the extreme early types and extreme late types at either end"

example, field data demonstrate a variation in performance (measured by cardiovascular endurance) of up to 26% depending on the time of day of tasks and an individual's circadian phenotype (Facer-Childs and Brandstaetter, 2015a). To put this data into perspective, a change in performance by 0.5% in the 2016 Olympics 100 m freestyle swimming event was the difference between fourth place and a gold medal, and a 2.5% increase in performance would have secured a gold medal, over Usain Bolt, for the last-placed runner in the 100 m sprint. Thus, there are likely gains to be made in elite performance from implementing chronobiological strategies that could make significant differences to sporting outcomes. Indeed, athletes may want to avoid mismatches between internal clock timing and real-life behaviours.

Schedule, sleep, and strategies

Elite athletes frequently face rigorous training and competition schedules including early morning rises and physically intense sessions. They also cannot control when, and where, the competition will be. Often, training, matches, or events can continue into the early evening, making it hard to switch off and settle before bed. Many elite athletes have documented that they have trouble "switching off" at bedtime following important sporting events. For instance, Paul O'Connell (a former professional rugby player) writes in his autobiography about the stress following games that impaired his sleep. In addition, elite athletes have personal lives and other commitments that can interfere with their sleep–wake cycles. Importantly, sleep can significantly affect training responses, performance, and risk of injury. For instance, the improvement in performance characteristics by NBA basketball players such as Andre Iguodala associated with improved sleep is remarkable (Berger, 2016).

Thus, it is quite clear that there is a need for elite athletes and their coaching teams to harness whatever advantage they can regarding their timing of behaviour, so that their sleep–wake and other activity schedules can assist in preparing optimally for peak performance. Although most competition schedules are rigid, training schedules can be carefully planned and managed by the high-performance team. Therefore, with the correct research base and expertise, manipulating these schedules might represent an opportunity to help in achieving optimal chronobiology.

Before factoring in circadian timing, however, a number of sleep hygiene recommendations could be considered, to assist athletes in their daily routines. A sleep schedule that is consistent in timing, of an adequate duration (for that individual), and of good quality is essential. The sleeping environment should have as little noise or light in order to promote sleep quality. Next, bright light, especially electronic device blue light should be limited in the hours preceding bed. Caffeine intake and meal timings should be carefully monitored and based on individual needs (Halson, 2008). Of course, all of these recommendations should be carried out in consultation with expert advice.

Sleep hygiene practice is often achievable with enough motivation. However, the consideration of chronobiology and circadian phenotype into elite athlete populations is practically more difficult and is a focus for future research. It requires some knowledge of the athlete's circadian rhythms and behaviours, which as previously discussed, can vary considerably between individuals and according to different schedules. Knowledge of circadian phenotype may be useful information but objective sleep monitoring for an adequate period as well as biological sampling is typically required in addition to continued one-on-one work with each individual. In the context of an under-pressure athlete, these factors may be viewed as burdensome; however, the benefits could outweigh the risk if performance was enhanced.

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"field data demonstrate a variation in performance (measured by cardiovascular endurance) of up to 26% depending on the time of day of tasks and an individual's circadian phenotype … 0.5% in the 2016 Olympics 100 m freestyle swimming event was the difference between fourth place and a gold medal"

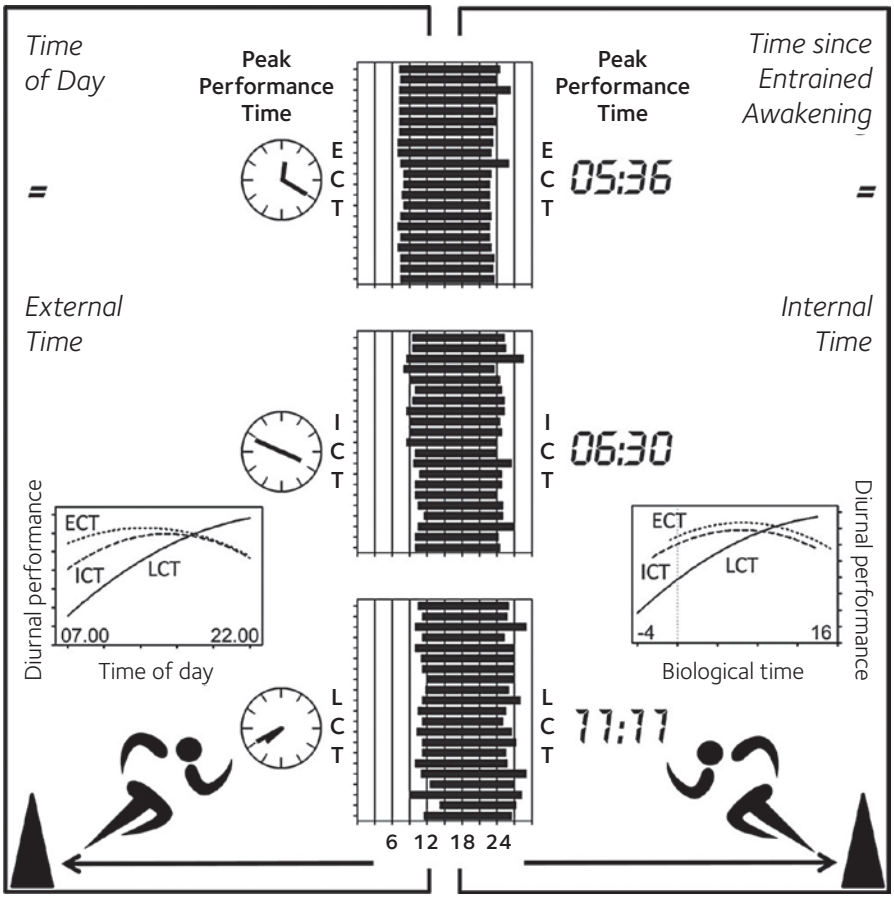

Figure 1. Graphical abstract from: All individuals were categorized as either early circadian phenotype (ECT), intermediate circadian phenotype (ICT), or late circadian phenotype (LCT); 28% of the individuals were ECTs (n = 34), 48% were ICTs (n = 58), and 24% were LCTs (n = 29). Reproduced from Facer-Childs & Brandstaetter, 2015a with permission from the authors. © 2015 Elsevier Ltd. All rights reserved.

The elite athlete as a special-risk traveller

A tangible example of behaving discordant with your internal timing is the phenomenon of jet lag from international travel. If one travels from London, England, to Melbourne, Australia, one arrives in a time zone and light–dark cycle operating 11 hours ahead. That is, touching down in Melbourne at 10:00 PM, you may feel quite alert, because your body is still entrained (i.e. the circadian system is adapted) to the light–dark cycle of London and thinks it is 11:00 AM. Due to the nature of elite athlete competitions, transmeridian travel is often inevitable. The subsequent circadian desynchrony that can lead to impaired performance is one problem that must be managed.

Some athletes travel days in advance so that they have time to adapt to a new time zone. Others may try to remain on their "home" time if their "home" timing of peak performance coincides with competition time. For instance, Formula 1 drivers from Europe may choose to stay on "home" time for the Singapore Grand Prix as the race time (night) in Singapore is closer to peak performance time (mid-afternoon) in Europe (Straka, 2011). In this instance, not only could circadian awareness improve performance, but when racing at high speeds, it could be key to preventing split-second lapses of attention resulting in grave injury. Similarly, North American West Coast football teams travelling east have an advantage when it comes to Monday night start times as their peak performance times may coincide with game times and this manifests in winning percentages (Jehue *et al.*, 1993). A significant disadvantage is presented to eastern teams who must travel west and more so for late start times because their "home" time could be after midnight (Roy and Forest, 2018).

Early and late types

It is important to note that one's circadian phenotype is on a spectrum, with the extreme early types and extreme late types at either end (Facer-Childs *et al.*, 2020). Although not everyone will be at either end, many people have a tendency towards one or the other. The composition of early and late types will vary slightly between populations and genders. For example, more females are early types and there are a higher proportion of late types amongst adolescents (Fischer

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et al., 2017). Individual differences will be seen in all populations, including educational settings, office-based jobs, safety-critical industries, the arts, and elite athletes. Yet, the reality is that most work–life schedules do not yet account for circadian phenotype.

We are all aware that feelings of alertness, productivity or sleepiness will vary throughout the day, but what is less commonly known is that these rhythms can differ significantly between early and late types. Research has shown that these groups have different diurnal rhythms in their mental performance, brain function, physical abilities and mood (Facer-Childs *et al.*, 2018, Facer-Childs *et al.*, 2019a).

Given the clear time-of-day variation in physiology, psychology and behaviour, schedules will seldom match these individual differences. Thus, in a typical day from 9:00 AM to 5:00 PM, students, workers and athletes will experience dips in performance and productivity that not only affect the individual, but can affect the team or people around them, all because they are forced to work at a time of day that their body does not agree with. For athletes, early morning training and rigid schedules may lead to the greater number of early types observed in some sports (Kunorozva *et al.*, 2012). Potentially, late types who typically compete in morning events may not appreciate that they can achieve higher levels of performance were the events scheduled later in the day and vice versa. Anecdotally, world records are typically broken in the late evening time, possibly due to television broadcasting prime times). One wonders whether more world records would be broken if athletes could compete at the optimal timing of performance as might be predicted by their circadian phenotype.

Future perspectives and consultant chronobiology

An elite athlete will have some insight or feeling into daily variations of when they feel or perform their best. However, with research-based evidence and expert consultants, an athlete can consider interventions to shift the timing of their biological rhythms to try and optimise performance (Facer-Childs *et al.*, 2019b). Research now demonstrates that there is a point in the day an athlete is likely to be achieving peak performance, based on circadian phenotype and time since entrained wake (Facer-Childs and Brandstaetter, 2015a). However, training and competition do not necessarily align with the athlete's internal timing. Therefore, there is motivation for a multidisciplinary team to work in conjunction with the athlete to shift their circadian timing to match that of the desired competition timing. For example, an early type may, on average, reach their peak performance at

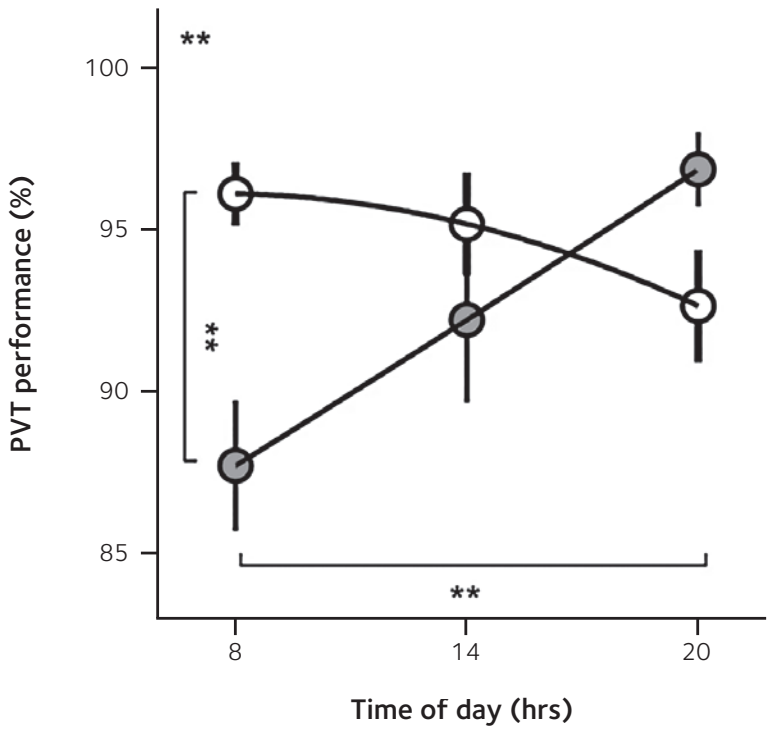

Figure 2. Diurnal variation in psychomotor vigilance (PVT) and PVT as a function of time since awakening. Clock time variation in PVT performance between ECTs (early circadian phenotype; white circles) and LCTs (late circadian phenotype; grey circles). PVT as a function of time since awakening for all subjects (n = 56). © Facer-Childs *et al.*, 2018 and is licenced under CC BY 4.0.

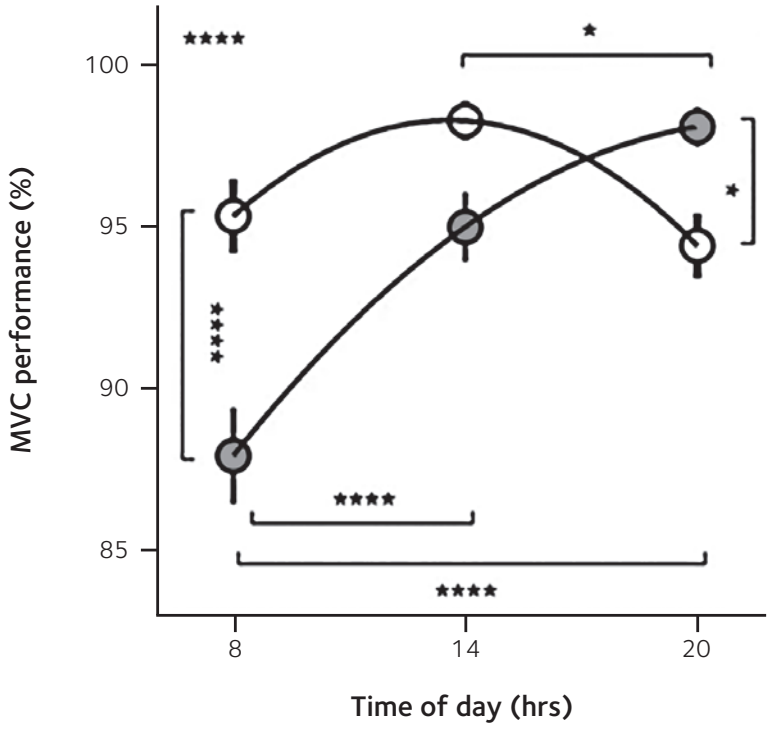

Figure 3. Diurnal variation in maximum voluntary contraction (MVC) and MVC as a function of time since awakening. Clock time variation in PVT performance between ECTs (early circadian phenotype; white circles) and LCTs (late circadian phenotype; grey circles). MVC as a function of time since awakening for all subjects (n = 56). © Facer-Childs *et al.*, 2018 and is licenced under CC BY 4.0.

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"North American West Coast football teams travelling east have an advantage when it comes to Monday night start times as their peak performance times may coincide with game times"

around 1:00 PM. However, their sport of choice may tend to be played in the evening. This athlete might, therefore, be motivated to delay their circadian rhythm. Whereas, for a late type who needs to perform their best earlier in the day, a phase advance of their circadian rhythm would be required.

On the extreme side, regardless of circadian phenotype, an athlete may need to participate in an international competition that is based in a time zone with 9 hours difference. This athlete/coaching team would be mindful to prepare for weeks in advance and consider techniques to adapt in consultation with a chronobiologist.

Practising good sleep hygiene, and interventions regarding circadian timing, has an exciting potential to improve recovery and increase performance in elite athletes (Samuels, 2008). Furthermore, awareness of these factors may have even broader implications beyond the individual. For team-based sports, it is possible that a coaching team may consider individual differences when planning schedules to manage team alertness and performance accordingly (Facer-Childs and Brandstaetter, 2015b).

It is important that the current research into chronobiology and sport is not taken out of context at this stage. Yes, differences in the peak performance of elite athletes will differ between early and late types. Similarly, there are techniques that can assist sleep health and help individuals to better adapt to travel, training or competition demands. However, importantly, this research is still in its infancy relative to other sports medicine fields. These techniques and potential interventions require carefully monitored practices by educated professionals with expertise in sleep and chronobiology. Athletes often have on-call or full-time physiotherapists, nutritionists and psychologists to provide expert opinions to assist the athlete in their quest for performance gains and competition achievement. Perhaps in the future, with increasing research and awareness in sleep and biological rhythms, sporting organisations will start to consider chronobiology consultants as part of their normal routine in preparation for elite competition.

For now, we hope that elite athletes together with their coaching and management teams can begin to increase their awareness of how factors such as sleep health, circadian phenotype, and chronobiology impact recovery and performance. Even a 1% change in performance could deliver the much-sought-after competitive advantage and deliver the gold. After all, timing is everything.

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Hypoxia and comparative physiology in humans

Diversity in haemoglobin and oxygen dissociation curve shifting

Michael J Joyner

Mayo Clinic, MN, USA

Paolo B Dominelli

University of Waterloo, ON, Canada

Haemoglobin is perhaps the most studied biologically important protein there is. In higher animals it is central to the transport of oxygen from the air to the tissues and thus imperative to exercise physiology. Haemoglobin levels can also be manipulated in athletes via altitude training and with nefarious practices like blood doping and the unauthorised use of erythropoietin. Subtle differences in the amino acid composition of the haemoglobin chains are also used in evolutionary and comparative biology to gain understanding about how the oxygen transport system adapts, via evolution, to environments like high altitude (Meir and Milsom, 2013). On the opposite end, sickle cell disease, caused by a change in one amino acid on the haemoglobin β chain, is also the sentinel example underpinning the concept of molecular medicine (Perutz, 1976). Haemoglobin is thus everywhere from the most molecular of research labs to surgical care on the battlefield.

Background

Beyond its biological properties, haemoglobin is embedded in medical physiology and biochemistry education. One of the key concepts in what is frequently taught is how four chains of haemoglobin interact as a unit to create the curvilinear oxygen haemoglobin dissociation curve (Fig. 1). The behaviour of this curve allows haemoglobin to be 90% saturated at relatively modest partial pressures of oxygen (60 mmHg) and provides a safety factor for hypoxia caused by moderate altitude or problems in the lung that limit the transfer of oxygen from the air to the blood. In several recent papers we have been exploring how left-shifted, high-oxygen affinity, haemoglobin affects how humans respond to hypoxia at rest and during exercise (Shepherd *et al.,* 2019; Dominelli *et al.,* 2019 & 2020). High-affinity haemoglobin means that for a given partial pressure, there is a higher oxyhaemoglobin saturation compared with typical haemoglobin (Fig. 1). Fundamentally, the high-affinity haemoglobin is able to bind or 'hold on' to oxygen more tightly.

Part of the standard teaching is that in response to moderate hypoxia there is a right shift or decrease in affinity of the oxygen haemoglobin dissociation curve. The narrative is that this right shift facilitates the unloading of oxygen at the tissues and defends the organism against hypoxia. This is a great story that tells important lessons about how proteins interact and also about allosteric feedback regulation of proteins by other molecules. In the case of haemoglobin, the allosteric regulation is most notably by 2-3 BPG (a byproduct of red blood cell glycolytic metabolism) and CO_2. However, some observations from comparative physiology tell a more complex story and our work on this topic in patients with rare forms of

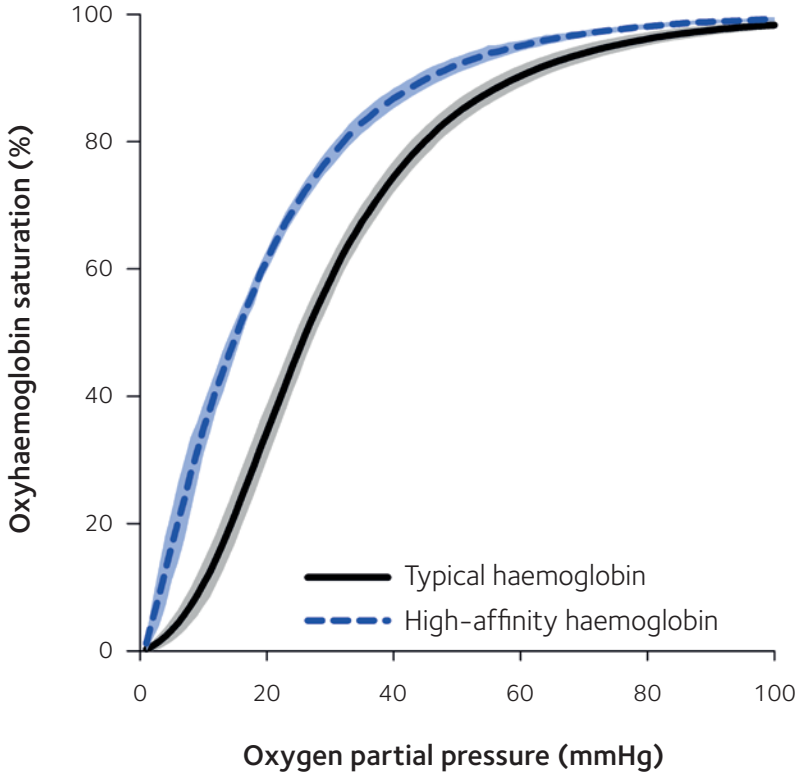

Figure 1. The relationship between oxygen partial pressure and oxyhaemoglobin saturation for typical haemoglobin and one high-affinity haemoglobin (Haemoglobin Malmo). The shaded area around each represents a typical range under resting conditions.

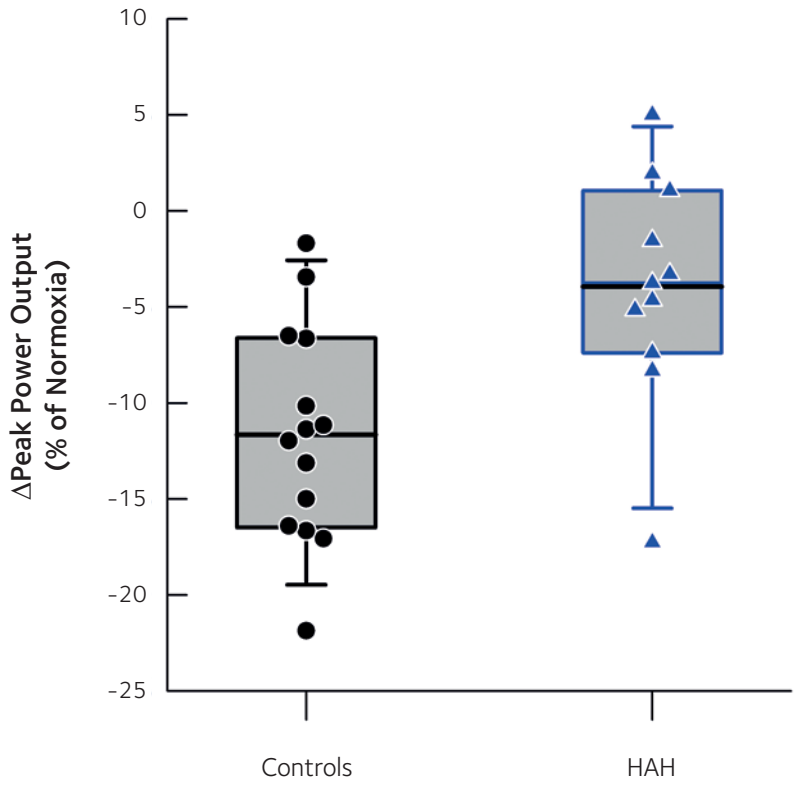

Figure 2. Box-whisker diagram of the normalised peak power output during maximal cycle ergometry hypoxia compared with normoxia in our controls (black dots) and left-shifted haemoglobin subjects (blue triangles).

haemoglobin that are left-shifted and have a higher than normal affinity for oxygen are challenging the simple version of this story.

The percolation

What is the rest of the story and where did our interest come from? In the early or middle 1990s one of us (MJJ) was attending a major scientific meeting in the USA. It was likely Experimental Biology then known as FASEB. Deciding to take a lesson from one of my medical school teachers Marlys Witte, who remains active in her middle 80s and is an advocate of gaining exposure about things you don't know about, I went to a comparative physiology talk on high-altitude animals. I have no idea who was giving that talk, but I do remember this incredible data about the bar-headed goose and how it flies over the Himalayan mountains. Birds have a cross-current lung that keeps the partial pressure of oxygen in their lungs closer to atmospheric than mammals, but the bar-headed goose also has left-shifted haemoglobin compared with lowland birds. The cross-current lung and air sac design of bird lungs means that there is little physiological dead space diluting atmospheric gas in bird lungs so that the partial pressure of oxygen presented to the pulmonary capillaries is similar to atmospheric. The left-shifted haemoglobin along with a drop in body temperature allows its haemoglobin to be more fully saturated when the partial pressure of oxygen is in the 30 – 40 mmHg range. To compensate for the higher affinity in arterial blood, unloading of oxygen at the muscles would then be facilitated by an increase in local temperature (Meir and Milsom, 2013). The result is these geese are able to perform extraordinary athletic feats in a severely hypoxic environment.

At the time I went to the talk it was also known or at least becoming known that exercising human muscles could extract more than 90% of the oxygen flowing across them during exercise, and that the mitochondria in skeletal muscles could work just fine at very low partial pressures of oxygen, a few mmHg or even less. This raised obvious questions about the right-shift story that can be summarised as: "If the muscles can extract everything, during hypoxic conditions like altitude, why not load the oxygen at the lung and let the muscles fend for themselves?" This question seemed especially germane to trained athletes because, among other things, several of the key adaptations to endurance training in skeletal muscles include more mitochondria and greater capillary density. Both of these adaptations should make it easier to get the oxygen from left-shifted haemoglobin.

The question sat there sort of percolating for about 20 years. It came up in overview presentations about oxygen transport and when I showed how much better the

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best athletes in the animal kingdom were compared to humans. I also knew from my clinical work that there were humans with rare haemoglobin variants that were left-shifted like the bar-headed goose and other high-altitude animals like llamas.

The realisation

Then a few years ago I learned I was eligible for a high-risk NIH grant so I applied. The first application on sex differences and blood pressure regulation was not deemed that high risk and was not funded. However, I resubmitted and figured that exploring right-shift dogma in rare patients might be high risk enough. This of course required me to have access to patients with rare haemoglobin variants and recruit them for studies. Fortunately I work at a major referral centre that does a lot of boutique diagnostic clinical testing including evaluating patients from all over the world for rare haemoglobinopathies. Jennifer Oliveira and James Hoyer run the clinical haematopathology lab at the Mayo Clinic and luckily they had an extensive list of patients and have been enthusiastic collaborators. My research nurse Shelly Roberts, is expert at patient recruitment and she felt that we would be able to find these patients and get them to volunteer for our studies. Discussions with the chair of our institutional review board indicated that ethical approval could be obtained with the proper oversight and safeguards. So we submitted what is, for the NIH, a very short (4-page) application outlining how we could test the right vs. left-shift question by interrogating every step of the oxygen transport cascade in patients with rare forms of haemoglobin using mostly a combination of exercise and hypoxia in conjunction with detailed physiological measurements. There were also just enough case reports from the 1970s and 1980s on exercise and hypoxia in patients with left-shifted haemoglobin to suggest the question was not completely out there and worth a more comprehensive evaluation (Shepherd *et al.,* 2019; Dominelli *et al.,* 2020).

So a good question, access to rare patients, a skilled staff and the right funding mechanism converged at the same time and the grant was funded. At about the same time, Paolo Dominelli was finishing his PhD at the University of British Columbia. Paolo was getting outstanding training on all things respiratory physiology in humans and how to study them and he contacted me about a postdoc. He was able to use the question to get a curiosity-driven and flexible NSERC postdoc grant from the Canadian government to collaborate. Although he was unfamiliar with human haemoglobin variants, he was very accustomed to altered-affinity haemoglobin in the comparative physiology literature as much of it occurred at the University of British Columbia. Thus, the stage was set.

The payoff

The main thing we have found so far is that when breathing 15% v/v oxygen (a similar partial pressure to atmospheric concentrations of oxygen at ~3,000 m of altitude) the exercise capacity of our left-shifted patients falls only about 4% compared with about 12% in our controls, thus the dogma presented in medical school physiology and biochemistry classes has limitations (Fig. 2). The higher exercise capacity in hypoxia appeared to come at the cost of greater metabolite buildup, namely lactate. We also found in separate experiments that these differences in exercise capacity were not related to alterations in the ventilatory response to hypoxia (Dominelli *et al.,* 2019).

Along these lines, as we dug deeper into everything about right and left-shifted haemoglobin we found 1960s papers about Dorset sheep in both the physiology and agriculture/animal science literature. Apparently this breed of sheep has animals with left-shifted haemoglobin. The animal physiologists in Australia were interested in the potential impact of the left-shifted variant haemoglobin on meat and wool production. They also did physiology experiments that included studying how right and left-shifted animals responded to either severe hypoxia or severe anaemia. The left-shifted animals did better during the hypoxic challenge while the right-shifted animals did better during anaemia (Dawson and Evans, 1966 & 1967).

This raises the possibility that when oxygen uptake at the lung is limiting, the best physiologic strategy is to load as much oxygen as possible at the lung and thus saturate the haemoglobin and hope extraction at the tissues is sufficient. By contrast, when O_2 content is reduced as in the case of anaemia without hypoxia then a right shift can work to deliver more oxygen to the tissues. These ideas may have therapeutic implications. For example, drugs that left-shift haemoglobin might be useful in limiting sickling in sickle cell disease and they might also be useful in extreme cases of hypoxia as in the adult respiratory distress syndrome. Several compounds are being developed and studied. A right-shifting drug (Efaproxiral) has been developed and its use explored in a number of situations where more oxygen unloading at the tissue might be useful. However, there are currently no approved uses for this drug and it is on the banned list for the purposes of sports doping.

Our larger story also shows the long and winding road to framing and addressing an interesting question, what can be learned when you spend time outside of your intellectual lane and the ongoing power of observations from comparative physiology and how a version of comparative physiology can be available in rare human patients.

"Haemoglobin is fundamental to exercise and clinical physiology"

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The bustling world of hormone dynamics

Endocrinology Theme Lead update

Paul Le Tissier

University of Edinburgh, UK

Tim Wells

Cardiff University, UK

It should go without saying that endocrinology is fundamental to physiology. Hormones are not only key mediators of homeostasis, but drive age-appropriate sea-changes in physiology within individuals. As such, they regulate and integrate all of the areas of physiology represented by the different Society themes. Recent advances in endocrinology have transformed the field. For example, identification of "new" hormones; realisation that well-established methodologies for quantification may be functionally misleading; and, identification of essential, previously unrecognised roles. These have provided dramatic new perspectives for endocrinologists. Rather than focus on these broader issues here, we would like to illustrate how these advances in endocrine research present challenges to our understanding, using examples from the growth hormone (GH) axis and its associated hormones.

The pattern of hormone secretion

Similar to many other hormones, it has long been recognised that GH secretion varies over multiple time scales, not only across the lifespan of an individual, but also with circadian and ultradian frequencies (Robinson and Hindmarsh, 2010; Steyn *et al.*, 2016). The different ultradian pattern between males and females has important consequences for hormone action. Frequent sampling (e.g. every 10 mins for at least 12 hours) is required to fully characterise the pattern of pulsatile GH secretion, but in common laboratory animals, such as mice, this is only possible over a very limited time period (Steyn *et al.*, 2011). New tools are now required to achieve temporal monitoring of hormone dynamics over these more extended periods. Use of nanomaterials and implanted devices utilising electrochemical or optical sensing of hormone levels or receptor activation holds promise for transforming the study of hormone dynamics, with the additional positive impact on the 3Rs – blood sampling no longer being necessary and longitudinal studies with animals acting as their own controls being possible.

Biologically active and inactive hormones

In common with many hormones, those of the GH axis circulate in both biologically active and inactive forms. This is exemplified by the gastric hormone ghrelin, which activates its receptor only when octanoylated, a reaction catalysed by ghrelin O-acyltransferase (GOAT) (Yang *et al.*, 2008). The expression of GOAT in several tissues expressing the ghrelin receptor suggests that there may be local regulation of hormone activity in these tissues (Hopkins *et al*, 2017). This has far-reaching implications, as the ability of the target tissue to regulate the activation state of the incoming hormone (Fig. 1) could have

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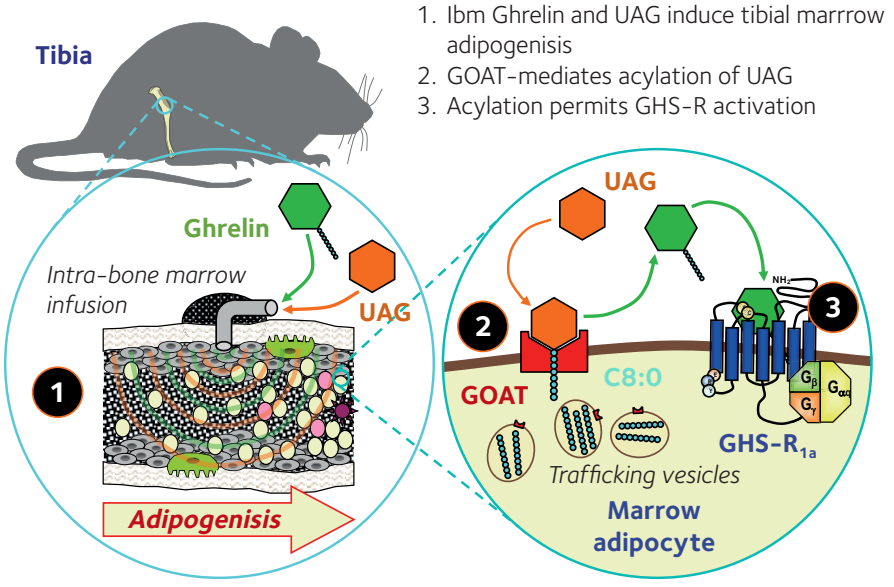

Figure 1. Target cell-activation of incoming hormones. 1. Intra-bone marrow (ibm) infusions of acylated and unacylated (UAG) ghrelin induce Adipogenesis in tibial bone marrow. 2. Ghrelin O-acyl transferase (GOAT) present in the plasma membrane of marrow adipocytes utilises medium-chain fatty acids (usually octanoic acid [C8:0]) stored in the adipocytes to acylate UAG. 3. Acylation to ghrelin permits activation of the ghrelin receptor ($GHS\text{-}R_{1a}$) (Hopkins *et al,* 2017).

"Integration of physiological processes for survival and reproduction requires the coordinated modification of multiple endocrine axes"

a profound influence over physiological action and pharmacological targeting.

Axis cross-talk

The complexity of any endocrine axis results in endocrinologists commonly (and understandably) focusing on their specific hormone system of interest. However, the integration of physiological processes for survival and reproduction requires the co-ordinated modification of multiple endocrine axes and cross-talk between axes. As physiologists we need to understand how the "wood" functions as a whole and not just the individual "trees" within it. The relationship of the GH axis with stress is an excellent example of this: studies to date, many contradictory, indicate that stress affects the secretion of GH but the consequences can vary depending on species, time frame and severity (Steyn *et al.*, 2016). This could also occur at multiple levels within the axis, with glucocorticoids, for example, both decreasing hypothalamic inhibition of GH secretion (Thakore *et al.*, 1994) and increasing transcriptional activity of GH-secreting somatotropic cells (Thiell *et al.*, 1993). The presence of several hormone receptors within a single cell type is, of course, one mechanism where integration of multiple axis functions can occur but there is increasing evidence for the modification of receptor responses by heterodimerisation. For example, the activity of melanocortin receptors, classically thought of as regulating stress and body weight have been shown to be modified by co-expression of the ghrelin receptor: the presence of both receptors in pituitary somatotropic cells may be a further mechanism integrating stress and the GH axes. Similar integration between the GH and gonadal axes may have important implications for current policy and health for those on reproductive steroid treatment.

Conclusion

These three features of the GH axis are common to many endocrine systems and indicate that continued study of individual hormones and their integrated interactions will continue to be a focus for many physiologists. Modulation of patterned secretion and hormone activation may underlie axis modification and interactions in response to altered physiological demand, such as pregnancy, as well as enhancing and preventing foetal exposure to circulating maternal hormones. Advances in these areas will require the adventurous combination of cross-disciplinary skills, from materials scientists, protein engineers, mathematicians and of course the endocrine physiologist to make sense of the wealth of data generated. Thus, although some may consider endocrinology to be a well-understood branch of physiology, a closer examination of factors such as hormone dynamics and local hormone activation, reveals a different picture. Understanding these more complex interactions will engender more informed policy development, more incisive diagnostic strategy and more appropriate patient treatment.

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Amazing Body: Creating colouring-in physiology for hospital patients

Lizzie Burns

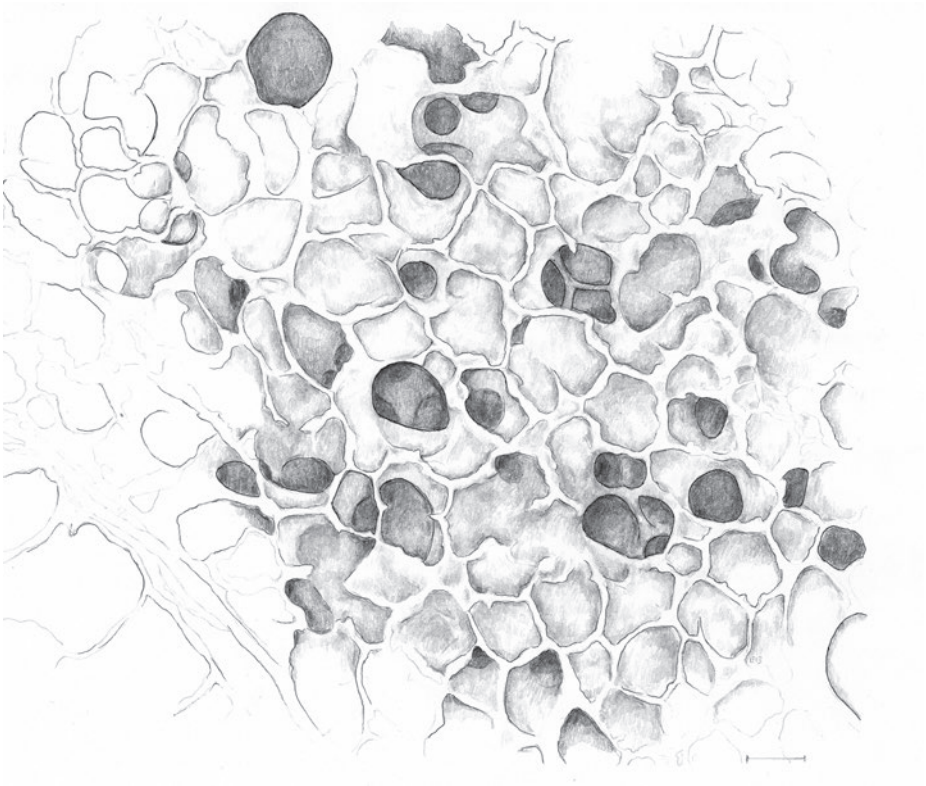
Science-based artist

This article was written before the COVID-19 pandemic situation in this country. These resources will soon be available on our website for anyone to learn about physiology in a creative way for relaxation at this challenging time. For more information please contact **edufunding@physoc.org**

Time is an opportunity. For those in hospital, time can be particularly precious. In contrast to children's wards, which are full of colour, toys and professionals encouraging play, there is little consideration for adult wards. To encourage change, I wrote a piece for the *British Medical Journal* raising the issue of boredom as an unacknowledged strain on adults in hospital and started the Anti-boredom Campaign, which has received support from both the media and health professionals.

Young adults are particularly prone to boredom, so how can we help? I work once a week as a Creative Specialist in University College Hospital in London with adults in oncology. The rest of the time, I work as a science-based artist and communicator. I have found many patients curious about science and seen how much adults enjoy colouring-in, a newly popular way to bring relaxation when focusing on reading is too difficult.

I approached The Physiological Society together with Members to propose a series of drawings for adult colouring-in. Sheets will be available as pdfs on The Physiological Society website for anyone to print out to give to those in hospital to help lift mood, spark curiosity, and encourage learning about our body – a remarkable entity even in disease. My aim is to create resources conveying physiology as dynamic, surprising, relevant, and crossing scales from micro to macro. Young adults with cystic fibrosis, who spend much time in hospital in isolation, will be offered the sheets. From sharing drawings recently with patients, one said, "It's good to know what you're made of – and it's beautiful." As well as being aimed at patients, the "Amazing Body" collection will become available for anyone to print out for public engagement events.



Alveoli: honeycomb structure within the lung. The scale represents 50 µm (around half the width of a human hair). This image is published under CC-BY-SA 4.0.

My background is in research; I hold a DPhil and research fellowship in cancer research from the University of Oxford. In 2002, I became a full-time science-based artist to communicate the beauty and wonder of science through artwork, as well as run workshops and events. I kept links with the Department for Physiology, Anatomy and Genetics at the University of Oxford and created illustrations for neuroanatomist Ray Guillery, for his final book *The Brain as a Tool*. It was this experience that encouraged me to create drawings that others bring to life by adding colour. Over the course of a year I am collaborating with The Physiological Society Members on a range of themes – how lungs stay inflated, hearing, the vascular system, and the generation of electricity in our bodies. Together we will come up with ideas as to what might be the basis for useful, interesting and engaging drawings to offer patients.

I started the project by meeting Peter Robbins and Keith Dorrington to consider lungs as a topic. I was fascinated by their interdisciplinary thinking – touching on engineering and maths – about the time-old question as to how lungs stay inflated. Together we looked at microscope images and considered whether crystal-like structures of surfactants, glimpsed through electron microscopy, may prevent collapse. I created a drawing based on electron microscope images of the intricate structures of alveoli. I also drew the beautiful mathematical structure (Schwarz P), which is a strong and stable structure and may be relevant at a molecular level as to how lungs do not collapse.

We involved origami to give people another layer of interaction. By folding a traditional "paper balloon" you can inflate a drawing of the microscopic honeycomb structure of alveoli using your own breath. Sheets will be given to people without a science background, so our aim is to provide enticing, uplifting images suitable for colouring-in.

Currently I'm working on drawings in collaboration with Andrew King based on

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hearing, Christopher Garland about the control of our vascular system, and Mark Dallas about the generation of electricity in our body at a molecular scale. Each topic offers so much creative potential and I'm looking forward to working on another four topics later in the year.

I would like to thank all the Members who have been so supportive, Ruth Charity from Artlink, the Art Coordinator from the Oxford University Hospital NHS Foundation.

Further reading

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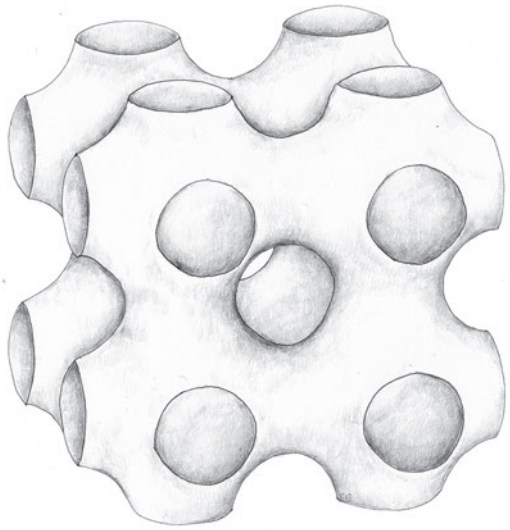

Schwarz P: mathematical shape may model how a liquid (surfactant) forms similar shapes that cannot collapse to ensure our lungs are open and flexible. This image is published under CC-BY-SA 4.0.

"My aim is to create resources conveying physiology as dynamic, surprising, relevant, and crossing scales from micro to macro"

Hibernation, lightning breath, and graphene moustaches: Members imagine possible futures of the human body

LEVEL UP HUMAN

Simon Watt

Presenter and producer, *Level Up Human*

Level Up Human is a comedy science podcast panel show. We answer a simple question: if you could redesign the human body, what would you change? With the support of a Physiological Society Outreach Grant we have been recording our second series.

The show is produced and presented by comedian Rachel Wheeley and me, Simon Watt, a biologist, comic and science presenter. The rest of the panel is made up of excellent guest experts who each have a chance to pitch one change they would make to the human body to make it better. We invariably delve into the complexities of our organs and what can go wrong with them, the tribulations caused by the legacy of evolution and some of the cutting-edge techniques used to tackle disease and disorder.

The suggestions included adding a zoom function to the eye, a live translation facility built into the ear and a green skin allowing us to photosynthesise. Our light format is tangent-friendly, and we are not afraid of discussing the silly and the sincere. I know I feel like I leave every recording having learnt something surprising. Our guests always benefit from being given a chance to think outside of the box, and discuss ideas with our live audience. As one of our panellists put it, we are "great practice for Radio 4."

The Physiological Society has given us access to a range of terrific Members who have helped make the podcast what it is, each drawing on their research for inspiration. Sarah Withers suggested the ability to change the colour of our adipose tissue, our fat. Specifically, she wanted us to be able to change white adipose tissue that acts mostly as energy storage, to brown adipose tissue that can generate heat. No more problems if you forget your jacket! She added that it could also give us a bonus ability; it might allow us, as it does for bears, to hibernate. We spent much of the rest of the episode discussing not just the physiology of fat but also what family events we might try to hibernate through.

Michael Preedy thought it would be great if we all had lightning breath. His reasoning was that it would allow us to produce our own nitric oxide, a molecule that plays a big role in maintaining cardiovascular health. And let's face it, it would look cool too.

Holly Shiels pitched a graphene moustache or nose hairs that would purify the air we breathe, keeping us safe from air pollution. Graphene is small and flexible, so it would easily fit. Particulate matter (PM) and polyaromatic hydrocarbons are released into the atmosphere when we burn fossil fuels. When they enter our body via the airways they can get into the blood and disrupt the electrical and contractile activity of our hearts. This can lead to various problems including arrhythmias and stroke. Graphene has already been inserted into air filters to help purify the indoor spaces.

Level Up Human has already completed a residency at the Barbican as part of their Life Rewired season, and recorded episodes for the Bluedot Music Festival. Later this year we will be going back to Bluedot Music Festival, Edinburgh Science Festival and Latitude Festival. You can listen to *Level Up Human* wherever you get your podcasts.

This article was written before the social distancing rules of COVID-19 were in place. The festivals mentioned in the article are cancelled for the foreseeable future, and instead Level Up Human runs remotely via the internet with guests from all over the globe. Visit **leveluphuman.com** to find out more.

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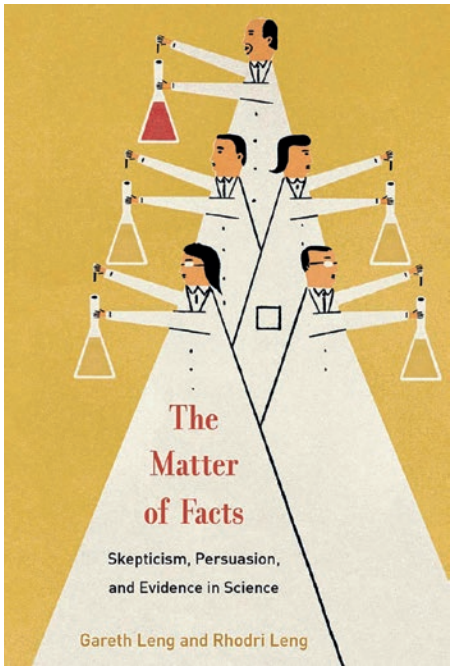
The Matter of Facts: Skepticism, Persuasion, and Evidence in Science

by Gareth Leng and Rhodri Ivor Leng

Philip Lewis

University Hospital of Cologne, Germany



Gareth Leng and Rhodri Ivor Leng
The MIT Press (2020)
ISBN: 9780262043885

"If a chapter was to be sampled at random, one's belief in the scientific process could be decimated"

Scientists are inherently involved in every part of the scientific process from research to peer review and the journal editor, to directing research institutions, and assessing prospective scientific staff. The job description can be summarised as something noble – the pursuit of new knowledge with utility. Yet, despite scientists' control over scientific processes and the virtuous job description, science is riddled with issues. The "publish or perish" paradigm, the injustice of impact factors, the autocratic reign of the p-value, publication bias, citation bias, and data replication issues are to name but a few. At the core of these problems is that scientists are only human and, thus, flawed. But, there is hope. *The Matter of Facts* by Gareth Leng and Rhodri Ivor Leng meets these issues head on from the combined perspective of an experimental scientist and sociologist (and, indeed, as father and son), respectively, and makes for a captivating read. As appropriately prefaced by the authors, this book is about "what it means to be a scholar" and that "science, as a human endeavour, is beset by all the flaws humans have, but is endowed with their virtues too".

The stall is set with references to the great scientific philosophers, Popper and Kuhn, and questioning how science progresses. What follows is a persuasive vivisection of the scientific process that – in my opinion at least – should make this book compulsory reading on all science courses.

This is not literature to be skimmed through lightly or wherein chapters could be sampled randomly. This is not because the prose is difficult to penetrate. On the contrary, the authors elegantly explain complex issues. But if a chapter was to be sampled at random, one's belief in the scientific process (especially the young scientist who is perhaps not yet battle-hardened by the wear and tear of criticism and rejection) could be decimated. Rather, this book should be read like one reads fiction, from start to finish, where science as the virtuous and heroic protagonist is doubted, shown to be flawed, and beaten down, before rising up to prevail in the end. But boy does science ever take a hammering in 21 of 25 chapters; and the authors are persuasive! Even the chapter titles – including "Is the Scientific Paper a Fraud …", "Exaggerated claims, semantic flexibility, and nonsense", and "Where are the facts?" – take no prisoners; but the arguments presented are fair.

The tenet of science that it is self-correcting is consistently found wanting – brought into question from various angles and with various examples in the various chapters. While their arguments are persuasive and important issues are highlighted, the conclusion that science is not self-correcting is, perhaps, incorrect. That the authors can identify cases where science is not self-correcting implies that these cases can be corrected now. Thus, in nice Popperian logic, the hypothesis that science is self-correcting is not falsified. Simply, there is a lag time to correction. Indeed, true peer review begins after publication.

Nonetheless, in many cases, my eyes were opened, particularly in regard to citation practices. For instance, that a "made-up" reference (Van der Geer J *et al.*, 2010. The art of writing a scientific article. *Journal of Science Communication* **163**(2) 51 – 59) intended to illustrate a journal's preferred style was apparently cited more than 480 times, including in some 79 journal papers, and to support the claim that the compound rutin affects blood pressure is so far beyond belief that one can only laugh (nervously). Indeed, I considered the possibility that the authors, as proof for the flaws in science that they state, included "made-up" examples to "test" if they would be taken at face value rather than the readers checking the primary evidence and interpreting for themselves. No such luck. And this is terrifically disheartening. (Disclaimer: I did not check 480 papers but I was quickly able to find a case of the "made-up" reference cited as evidence of rutin affecting capillary permeability).

In the end, it is clear that science is no paragon. There are innumerable pitfalls to be aware of. Importantly, though, at no point do the authors completely write science off. The hero prevails in the end. How do we know? Simple – science progresses.

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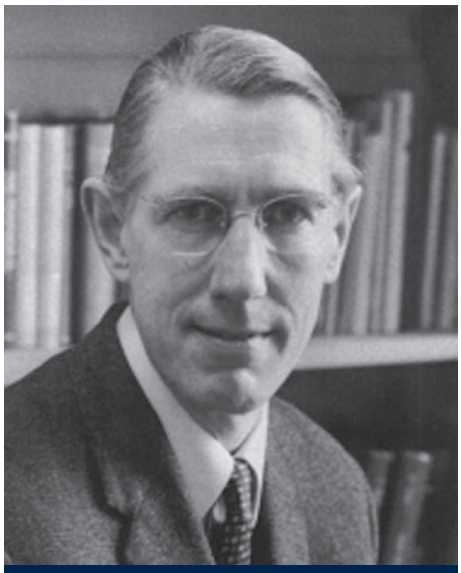
Obituary: Peter Bryan Conrad Matthews 1928 – 2020



Peter Bryan Conrad Matthews

Peter Matthews was born 23 December 1928, the son of Bryan and Rachel Matthews, both of whom were physiologists. In Cambridge he attended King's College Choir School where, to quote Peter himself, he studied "Latin and maths, Latin and maths" but no science, before moving to Marlborough College in 1942 where his interest and aptitude in science were nurtured.

He won a scholarship to King's College Cambridge to read Natural Sciences (in chemistry, physics, and physiology), and graduated with a first in 1946. During his final year at Cambridge, Peter became friends with Alan Turing who had returned to his old room in King's College as a Fellow, following his still secret war work at Bletchley Park. Despite Peter's professed lack of mathematical ability, he recalls Alan showing him a mechanical differential analyser that Alan was using in his work on the mathematics of pattern formation in animals, such as the stripes of the zebra. One could scarcely have had an earlier introduction to computing!

A year later, he moved to Mill Hill on an MRC studentship where he worked on Pacinian corpuscles under John Gray, confirming their rapidly adapting nature. Pacinian corpuscles are found in many connective tissues and around joints, and had been identified, wrongly, by Edgar Adrian as slowly adapting. Actually they are so rapidly adapting that they are highly sensitive to vibration and react best to a high-frequency mechanical stimulus.

Peter then returned to Cambridge for a year of preclinical study before moving to Oxford, where he spent the rest of his academic career, for the clinical components of his medical degree.

During his clinical studies, Peter became intrigued by an observation of the neurologist Francis Walshe that an injection of the anaesthetic procaine into the muscles of Parkinson's patients would relieve their spasticity. He doubted Walshe's interpretation that this was due to the preferential block of the large afferents from muscle spindles. Instead he thought that if procaine preferentially blocked the small efferent gamma fibres a similar relief of spasticity would occur. The gamma fibres had recently been identified by Leksell as purely fusimotor in action, implying their exclusive distribution to muscle spindles. Peter was soon able to confirm his idea, initially working alone in 6-month periods between house jobs, and later in collaboration with Geoffrey Rushworth.

> "Peter retired in 1996, a year after becoming an Honorary Member of The Society"

In 1956 he was appointed University Demonstrator in the Department of Physiology, then headed by EGT Liddell. In the same year he also married Margaret Rosemary Blears, who published numerous papers on autonomic neurons under her married name. The marriage was to last throughout Peter's life. In 1961 he was appointed University Lecturer in Physiology.

Peter's experience with procaine led on to his seminal work in which he identified A1 and A2 types of muscle afferents, discovered by his father, as being muscle-spindle primary and secondary endings respectively. He also showed the complex nature of the primary ending's response to stretch, which exhibited both phasic (or dynamic) and tonic (or static) components as compared to the simpler, more or less purely tonic response of the secondary endings. He found that the phasic component of the primary response is such that the ending is highly sensitive to vibration, which he was to exploit most productively in later studies on reflex actions. And he divided gamma fibres into two functional categories according to their effects on the primary ending's response, naming them dynamic and static according to whether they enhanced the phasic or tonic components. He conducted all of this work alone or in collaboration with few others, notably Michael Brown, Robin Harvey, Jan Jansen, Alan Crowe, Dick Stein, and Manuel Hulliger.

Peter published several highly influential reviews, most importantly The Physiological Society's Monograph "Mammalian Muscle Receptors and their Central Actions" in 1972. The same year also saw the publication of what was to become his most cited paper to date: "Contribution of muscle afferents to kinaesthesia shown by vibration induced illusions of movement and by effects of paralysing joint afferents." In the beautifully conceived and conclusive experiments conducted on themselves, Peter, Guy Goodwin and Ian McCloskey showed that muscle afferents contributed to conscious position sense, in contrast to the then widely accepted view that they played no role in conscious perception. From then on, most of Peter's work concerned reflex actions in motor control, extending the insights gained from single-unit work in the cat to human reflex function.

Peter retired in 1996, a year after becoming an Honorary Member of The Society, but continued publishing for some years afterwards, most recently an introductory review to the proceedings of my own retirement symposium in 2014. He attended together with his wife, despite having suffered a non-dominant stroke about 10 years earlier. During the scientific sessions of the meeting he displayed the same sharpness of intellect and command of his subject that was familiar to those attendees of my generation.

Peter died 2 March 2020 aged 91. He is survived by his wife Margaret, their son Hugh, and daughter Clare.

Written by Bob Banks (Durham University).

https://doi.org/10.36866/pn.118.49

Obituary: Michael Berridge 1938 – 2020

Michael Berridge

Mike Berridge, pioneer, and mentor of the calcium (Ca^{2+}) signalling field, died on 13 February 2020. His discovery that the signalling molecule inositol trisphosphate (IP_3) links receptor activation to generation of intracellular Ca^{2+} signals and his talent for seeing the big picture transformed our understanding of cell regulation.

Mike was born in Southern Rhodesia (now Zimbabwe) in 1938, and his love of African wildlife, especially elephants, was channelled by an inspirational teacher into a life-long study of biology. He graduated from the University College of Rhodesia and Nyasaland in Salisbury (Harare), and in 1961 he was awarded a Commonwealth PhD Scholarship in the Department of Zoology, University of Cambridge under the father of insect physiology, Sir Vincent Wigglesworth (known as VBW). Here, Mike investigated the metabolic and excretory mechanisms of the Malpighian tubules of an insect pest, the cotton stainer. He became interested in how these processes were regulated by hormones; and he imbued the VBW ethos of working at the bench without a large group, with simple equipment and elegantly decisive experiments, and with an astonishing breadth of knowledge across biology.

At the University of Charlottesville, Virginia and then Case Western Reserve, Cleveland, Mike pursued his interests in hormone action and, with no cotton stainers available, he switched to blowflies. While dissecting their Malpighian tubules, he noticed two long transparent structures, the salivary glands, and showed that 5-HT stimulated them to secrete the prodigious amounts of saliva that allow the fly to feed before it can be swatted.

During those early years with fly salivary glands, Mike's neighbour at Cleveland was Ted Rall, who had isolated cAMP and helped develop the "second messenger" concept with Earl Sutherland. With advice from Ted, Mike showed that cAMP also stimulated salivary secretion and that inhibitors of cAMP phosphodiesterases potentiated the effects of 5-HT. The work, published when a single decisive figure was enough for *Science*, led John Treherne to invite him to join the Agricultural Research Council Insect Physiology Unit back in the Department of Zoology, Cambridge.

Here, working with William Prince, Mike used electrophysiology to show that two different 5-HT receptors controlled secretion; one receptor coupled to formation of cAMP, and the other somehow required Ca^{2+}. They also observed, in 1973, that agonists of salivary secretion evoked oscillations in the Ca^{2+}-dependent transepithelial currents, the frequency of which increased with agonist concentration. The observation was both prescient – it was another 13 years before Peter Cobbold reported frequency-encoded Ca^{2+} oscillations in single hepatocytes, and it inspired Mike's interests in the spatio-temporal organisation of Ca^{2+} signals. Some thought Mike's early interest in fly spittle to be arcane, but from 1975 onwards his reviews and expanding research base demonstrated his ability to range widely and thoughtfully across cell biology, and they presaged his later career when well-crafted reviews and lectures brought cell signalling to diverse audiences.

Although salivary glands needed extracellular Ca^{2+} for sustained secretion, they could transiently secrete in response to stimulation of 5-HT receptors without it. These observations implied that there was an intracellular source of Ca^{2+} that could initiate salivary secretion; but how did the 5-HT receptor stimulate release of Ca^{2+} from this intracellular store? Here, the blowfly salivary gland and Mike's inspired use of it, proved decisive. Bob Michell, drawing on earlier work from the Hokins and others, had published a landmark review in 1975 in which he argued that breakdown of phosphoinositides in the plasma membrane, the so-called 'PI effect', was causally linked to Ca^{2+} signalling. The gland is permeable to inositol and, during production of copious amounts of saliva, inositol is progressively lost, consequently leading to a reduced supply of inositol lipids on which the PI effect depends. Mike showed that during sustained stimulation, both the Ca^{2+}-dependent secretion and the PI effect evoked by 5-HT were restored by addition of exogenous inositol. This work, along with other experiments, indicated that Ca^{2+} signalling was downstream of the PI response. He further showed, in what he described as his "eureka moment", that inositol 1,4,5-trisphosphate (IP_3) was the first water-soluble product of the PI effect. Hence, Ca^{2+} signals required the PI effect, and IP_3 was the prime candidate for causing Ca^{2+} release from intracellular stores. By chance, Mike had recently heard Irene Schulz talk about her use of permeabilised pancreatic acinar cells to monitor Ca^{2+} fluxes, and Robin Irvine and Rex Dawson, phosphoinositide world experts, were only six miles away at Babraham. With Robin's stock of IP_3, Irene's cells and her post-doc Hans-Peter Streb performing the experiments, it was straightforward to show that IP_3 selectively evoked Ca^{2+} release from a non-mitochondrial Ca^{2+} store. The report in *Nature*, with just three simple panels and no supplementary materials, became a citation classic and its key findings were quickly confirmed in numerous cell types.

With IP_3 firmly established as a ubiquitous intracellular messenger, Mike's attention moved towards the physiological consequences of its activities. Mike collaborated with Roger Moreton in Zoology to build an imaging system, based on a design by Roger Tsien, to measure Ca^{2+} signals in living cells. Such systems are now commonplace, but in those days it was quite an undertaking, and it paved the way to exploring the intracellular complexity of Ca^{2+} signals. Mike was fascinated by how Ca^{2+} signals are organised in time and space, how the complexity of their organisation regulates specific cellular responses, and what happens when IP_3-evoked Ca^{2+} signals go wrong.

In later years, Mike's considerable impact came through his creative assimilation of observations from diverse areas of biology, and not least from the many posters to which he paid close attention at every meeting. In influential reviews and lucid talks, each invariably illustrated with instructive cartoons, Mike developed new ideas around the actions of Li^+, mechanisms for oscillatory Ca^{2+} signals, and tied IP_3-evoked Ca^{2+} signalling seamlessly into diverse areas of physiology and pathology. The trademark clarity of his diagrams formed the basis of his authoritative

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online textbook on cell signalling, Cell Signalling Biology, which can be viewed online.

Mike took particular interest in the careers of young scientists, and we were fortunate, as his PhD students, to have benefitted directly from that. Mike enjoyed seeing enthusiastic young scientists advancing in their careers. He established an annual prize to support young scientists, and the European Calcium Society established a lecture in his honour. He was elected a Fellow of The Royal Society in 1984, as a foreign member of the US National Academy of Sciences in 1999, and he was a founding member of the Academy of Medical Sciences. Among his many international prizes were the King Faisal International Prize, Louis-Jeantet Prize, Gairdner Award, Lasker Award, Wolf Prize, Shaw Prize and Royal and Croonian Medals from The Royal Society. He was knighted for his services to science in 1998.

Throughout his career, Mike was supported by his wife Sue, who, with their son and daughter, Paul and Rozanne, shared the excitement of his discoveries and provided the rock on which Mike built his exceptional career.

In addition to his own critical contributions to cell biology, Mike presided, in the most gentlemanly of ways, over the field of the Ca^{2+} signalling, and he inspired generations of future signallers. The three authors, all of whom continue to work on Ca^{2+} signalling, express their enormous gratitude for his mentorship, support and wonderful friendship throughout their careers.

Written by Martin Bootman (The Open University), Antony Galione (University of Oxford) and Colin Taylor (University of Cambridge).

Obituary: David Arthur Jones 1943 – 2020

David Arthur Jones

David was elected a Member of The Physiological Society in 1978, was a Distributing Editor for *The Journal of Physiology* (1993 – 1999), and was involved in setting up the Human Physiology Interest Group, now the Human, Environmental, and Exercise Physiology Theme. He graduated in 1965 from Birmingham University in Medical Biochemistry and then took up an MRC training studentship at the Institute of Psychiatry in London, first with Richard Rodnight and then as a research assistant to Henry McIlwain. In 1971, he became a Research Fellow at the Royal Postgraduate Medical School, Hammersmith Hospital, with Richard Edwards and David Hill. David Jones, with Pat Merton and later Brenda Bigland-Ritchie, were the first to describe "high-frequency" and later also "low-frequency" fatigue, and among the first to use electrical stimulation to differentiate between central and peripheral fatigue.

In 1976, at the Department of Medicine, UCH, working with Malcolm Jackson on zinc absorption, they were amongst the first to use stable isotopes in humans. With Di Newham he showed that delayed onset muscle soreness (DOMS) is not the result of muscle fibre damage, but probably due to connective tissue changes. With Joan Round he studied muscle growth during childhood and adolescence. With Olga Rutherford he was one of the first to use ultrasound and CT to study training-induced changes in muscle architecture.

Through teaching on the MSc course about muscles run by Roger Woledge, Nancy Curtin and Sally Page at UCL, David first worked with Tony Sargeant leading to a long-term collaboration with many Dutch colleagues, among them Arnold de Haan. They showed that fatigue is not only associated with loss of strength but also slowing. Experiments at UCH with magnetic resonance spectroscopy demonstrated that the loss of function with fatigue is not necessarily caused by the accumulation of lactate or H^+.

In 1993, he became Head of the School of Sport and Exercise Sciences at Birmingham University, where he found that a major factor limiting prolonged exercise is an increase in both core and skin temperature. With Alison McConnell and Lee Romer he showed the value of respiratory muscle training. Most intriguing was the observation that directly infusing glucose had no beneficial effect, while rinsing the mouth with carbohydrates did improve performance. Using fMRI, they found that carbohydrates influenced, via sensors in the mouth, parts of the brain associated with motivation.

In 2004, he was appointed at Manchester Metropolitan University, where, with Hans Degens, he found that accumulation of inorganic phosphate depresses force production and that low levels of calcium may contribute to slowing of the muscle fibre shortening. At Manchester he also studied the variable response of individuals to both strength and endurance training. His work on ageing expanded into a large European survey in collaboration with Marco Narici and Jamie McPhee, which led to a study of the motor unit changes with ageing. David continued to be active right up until his premature death, making major contributions to research with collaborators in Lithuania, and in Thailand with his wife Chulee Ubolsakka-Jones on strategies to improve respiratory function in a range of patient groups.

His teaching was filled with humour and incredible patience. I myself (Hans Degens) must have caused him at times exasperations when he tried explaining to me the intricacies of cross-bridge kinetics, not once, but repeatedly. His saying that "old men are like young women" filled us with surprise and laughter, but he clarified he was not talking about beauty, but rather about the fact that the muscle mass in old men resembles that of young women. David was a generous man, a man with a listening ear, who was always happy to lend support where he could. We have lost a great friend.

David was predeceased by his first wife in 1993. He is survived by his son Luke from that marriage and by his second wife Chulee.

Written by Hans Degens (Manchester Metropolitan University), Tony Sargeant (retired), Di Newham (King's College London), Jamie McPhee (Manchester Metropolitan University) and Malcolm Jackson (University of Liverpool).

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